09/579,894

(FILE 'HOME' ENTERED AT 17:09:57 ON 01 MAY 2002)

FILE 'CAPLUS, EMBASE, BIOSIS, MEDLINE, WPIDS' ENTERED AT 17:10:38 ON 01 MAY 2002

L1 177 S (SAKSELA, K? OR SAKSELA K?)/AU,IN
L2 21 S (HIIPAKKA, M? OR HIIPAKKA M?)/AU,IN
L3 17 S L1 AND L2
L4 5 DUP REM L3 (12 DUPLICATES REMOVED)
L5 1993 S (VARIABLE OR RT)(2A)(LOOP?)
L6 181 S L1 OR L2
L7 13 S L5 AND L6
L8 4 DUP REM L7 (9 DUPLICATES REMOVED)
L9 16 S L5 (10A) (LIBRAR? OR RANDOM? OR COMBINATORIAL? OR PHAGE? OR
L10 8 DUP REM L9 (8 DUPLICATES REMOVED)
L11 180 S (SH3)(3A)(DOMAIN?)(10A) (LIBRAR? OR RANDOM? OR COMBINATORIAL?
L12 101 S (SH3)(3A)(DOMAIN?)(5A) (LIBRAR? OR RANDOM? OR COMBINATORIAL?
L13 46 S (SH3)(3A)(DOMAIN?)(5A) (RANDOM? OR COMBINATORIAL? OR PHAGE? O
L14 23 DUP REM L13 (23 DUPLICATES REMOVED)

FILE 'STNGUIDE' ENTERED AT 17:23:15 ON 01 MAY 2002

FILE 'CAPLUS, EMBASE, BIOSIS, MEDLINE, WPIDS' ENTERED AT 17:23:51 ON 01 MAY 2002

FILE 'STNGUIDE' ENTERED AT 17:25:21 ON 01 MAY 2002

FILE 'CAPLUS, EMBASE, BIOSIS, MEDLINE, WPIDS' ENTERED AT 17:25:57 ON 01 MAY 2002

FILE 'STNGUIDE' ENTERED AT 17:27:16 ON 01 MAY 2002

FILE 'CAPLUS, EMBASE, BIOSIS, MEDLINE, WPIDS' ENTERED AT 17:27:37 ON 01 MAY 2002

FILE 'STNGUIDE' ENTERED AT 17:31:01 ON 01 MAY 2002

FILE 'CAPLUS, EMBASE, BIOSIS, MEDLINE, WPIDS' ENTERED AT 17:32:08 ON 01 MAY 2002

FILE 'STNGUIDE' ENTERED AT 17:32:41 ON 01 MAY 2002

=>

L14 ANSWER 20 OF 23 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 6
AN 1994:624597 CAPLUS
DN 121:224597
TI Identification of a Src **SH3 domain** binding motif by screening a **random** phage display library
AU Cheadle, Christopher; Ivashchenko, Yuri; South, Victoria; Searfoss, George H.; French, Stephen; Howk, Richard; Ricca, George A.; Jaye, Michael
CS Dep. Molec. Biol., Rhone-Poulenc Rorer Central Res., Collegeville, PA, 19426, USA
SO J. Biol. Chem. (1994), 269(39), 24034-9
CODEN: JBCHA3; ISSN: 0021-9258
DT Journal
LA English

=> d 20 ab

L14 ANSWER 20 OF 23 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 6
AB A phage display library was constructed in the filamentous bacteriophage fuse5. The library was made by inserting a degenerate oligonucleotide which encodes 15 variable amino acids into the N-terminal region of the phage gene III protein. This library, contg. >107 different phage, was screened with a glutathione S-transferase (GST) fusion protein contg. the Src homol. 3 (Src SH3) domain and a protein kinase A phosphorylation site (GST/PKA/Src SH3). A family of proline-rich sequences was isolated following 4 cycles of enrichment and amplification. Phage contg. these sequences specifically bind to the GST/PKA/Src SH3 protein but not to GST/PKA only. A comparison of the inferred amino acid sequence of the different phage clones revealed a consensus sequence, RPLPXXP, which conforms to a Src SH3 domain binding motif identified independently during an affinity screen of a .lambda.-lox mouse embryo cDNA library using a 32P-labeled Src SH3 protein fragment as the probe. Peptides based upon the 7-amino acid SH3 binding domain core motif displayed strong binding to both the Src and to the Fyn SH3 domains, but failed to bind to the SH3 domain of p21Ras-GTPase-activating protein (Ras-GAP) and other proteins. Further screening of the phage display library will be a useful tool for the rapid identification of addnl. SH3 domain binding sequences and will also help to establish the essential core motifs that define the specificity of interactions among the diverse proteins contg. SH3 domains and those contg. SH3 binding motifs.

=>

L14 ANSWER 12 OF 23 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 4
AN 1998:374512 CAPLUS
DN 129:158791
TI Mapping the specificity of **SH3 domains** with **phage**-displayed **random**-peptide libraries
AU Sparks, Andrew B.; Rider, James E.; Kay, Brian K.
CS Curriculum in Genetics and Molecular Biology, University of North Carolina at Chapel Hill, NC, USA
SO Methods Mol. Biol. (Totowa, N. J.) (1998), 84(Transmembrane Signaling Protocols), 87-103
CODEN: MMBIED; ISSN: 1064-3745
PB Humana Press Inc.
DT Journal
LA English

=> d 12 ab

L14 ANSWER 12 OF 23 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 4
AB This article describes the construction and screening of phage-displayed random-peptide libraries, with an emphasis on the application of these methods to the anal. of SH3-ligand preferences.

L14 ANSWER 16 OF 23 CAPLUS COPYRIGHT 2002 ACS
AN 1996:751514 CAPLUS
DN 126:16497
TI Proteins containing SH3 domain(s) and methods for identifying functional domain-containing proteins and kits for drug discovery
IN Sparks, Andrew B.; Hoffman, Noah; Kay, Brian K.; Fowlkes, Dana M.; Mcconnell, Stephen J.
PA Cytogen Corporation, USA; University of North Carolina At Chapel Hill
SO PCT Int. Appl., 172 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 2

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9631625	A1	19961010	WO 1996-US4454	19960404
	W: AL, AM, AU, AZ, BB, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, IS, JP, KG, KP, KR, KZ, LK, LR, LS, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, TJ, TM, TR, TT, UA, UZ, VN, AM, AZ, BY, KG				
	RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
	US 6309820	B1	20011030	US 1996-630915	19960403
	AU 9653821	A1	19961023	AU 1996-53821	19960404
	AU 711141	B2	19991007		
	EP 833941	A1	19980408	EP 1996-910696	19960404
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
	JP 11509172	T2	19990817	JP 1996-530406	19960404
PRAI	US 1995-417872	A	19950407		
	US 1996-630915	A	19960403		
	WO 1996-US4454	W	19960404		

=> d 16 kwic

L14 ANSWER 16 OF 23 CAPLUS COPYRIGHT 2002 ACS
AB . . . of the method and of the polypeptides identified are described, including their use in assay kits for drug discovery. Using **SH3** **domain**-binding peptides from **combinatorial** libraries as recognition units, a series of mouse and human cDNA expression libraries were screened. Sixty-nine of the 74 clones. . .

IT Peptides, analysis
RL: ANT (Analyte); BUU (Biological use, unclassified); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(**combinatorial** libraries; proteins contg. **SH3** **domain**(s) and methods for identifying functional domain-contg. proteins and kits for drug discovery)

=>

09/579,894

WEST Search History

DATE: Wednesday, May 01, 2002

Set Name side by side	Query	Hit Count	Set Name result set
	DB=USPT,PGPB,JPAB,EPAB,DWPI,TDBD; PLUR=YES; OP=OR		
L12	L10 and SH3	0	L12
L11	L10 and Nef	0	L11
L10	licentia	16868	L10
L9	l7 near7 (librar$ or random$ or combinatorial$ or artificial$ or modify or modified)	2	L9
L8	L7 and NEF	1	L8
L7	(RT)near2(loop$)	66	L7
L6	L5 and Nef	9	L6
L5	HIIPAKKA or SAKSELA,	137	L5
L4	RRT-SH3	1	L4
L3	(SH3)near3(RT-loop$)	2	L3
L2	L1 and RT$	15	L2
L1	SH3 and Hck	71	L1

END OF SEARCH HISTORY

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FILE 'HOME' ENTERED AT 13:52:37 ON 09 APR 2001

=> file cas

'CAS' IS AN AMBIGUOUS FILE OR CLUSTER NAME
CASLINK - Linked CAS files (Predefined Search Sequences)
CASRNS - CAS Registry Numbers Cluster
CA - The Chemical Abstracts File for 1967-present
CASREACT - The Chemical Abstracts Reaction Search Service
ENTER FILE OR CLUSTER NAME (IGNORE):ca

	SINCE FILE ENTRY	TOTAL SESSION
COST IN U.S. DOLLARS		
FULL ESTIMATED COST	0.75	0.75

FILE 'CA' ENTERED AT 13:55:23 ON 09 APR 2001
USE IS SUBJECT TO THE TERMS OF YOUR STN CUSTOMER AGREEMENT.
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FILE COVERS 1967 - 5 Apr 2001 VOL 134 ISS 16
FILE LAST UPDATED: 5 Apr 2001 (20010405/ED)

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```
=> s hck

L1          259 HCK

=> s l1 and SH3

          2186 SH3
L2          54 L1 AND SH3

=> s l2 and library

         44972 LIBRARY
L3            7 L2 AND LIBRARY

=> d l3 1-7 ibib ab

L3   ANSWER 1 OF 7  CA  COPYRIGHT 2001 ACS
ACCESSION NUMBER:        134:159189  CA
TITLE:                   SH3-binding peptides specific for the
                         Src-family of proteins
INVENTOR(S):             Sparks, Andrew B.; Kay, Brian K.; Thorn, Judith M.;
                         Quilliam, Lawrence A.; Der, Channing J.; Fowlkes,
Dana
                         M.; Rider, James E.
PATENT ASSIGNEE(S):      University of North Carolina at Chapel Hill, USA;
                         Cytogen Corp.
SOURCE:                  U.S., 150 pp., Cont.-in-part of U.S. Ser. No.
483,555.
                         CODEN: USXXAM
DOCUMENT TYPE:           Patent
LANGUAGE:                English
FAMILY ACC. NUM. COUNT:  3
PATENT INFORMATION:

     PATENT NO.        KIND  DATE          APPLICATION NO.  DATE
     ---------------   ----  --------      ---------------  --------
     US 6184205         B1   20010206      US 1996-602999   19960216
     CA 2195629         AA   19960208      CA 1995-2195629  19950724
     CA 2246378         AA   19970821      CA 1997-2246378  19970214
     WO 9730074         A1   19970821      WO 1997-US2298   19970214
         W:  AL, AM, AU, AZ, BA, BB, BG, BR, BY, CA, CN, CU, CZ, EE, GE, HU,
             IL, IS, JP, KG, KP, KR, KZ, LC, LK, LR, LT, LV, MD, MG, MK, MN,
             MX, NO, NZ, PL, RO, RU, SG, SI, SK, TJ, TM, TR, TT, UA, UZ, VN,
             YU, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
         RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR,
             IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML,
             MR, NE, SN, TD, TG
     AU 9722723         A1   19970902      AU 1997-22723    19970214
     AU 726263          B2   20001102
     EP 897392          A1   19990224      EP 1997-905952   19970214
         R:  AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,
             IE, FI
     JP 2000506522      T2   20000530      JP 1997-529492   19970214
PRIORITY APPLN. INFO.:                     US 1994-278865   19940722
                                           US 1995-483555   19950607
                                           US 1996-602999   19960216
                                           WO 1997-US2298   19970214

AB   Peptides having general and specific binding affinities for the Src
homol.
     region 3 (SH3) domains of proteins are disclosed in the present
     invention.  In particular, SH3 binding peptides have been
     isolated from phage-displayed random peptide libraries which had been
     screened for isolates that bind to bacterial fusion proteins having an
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SH3 domain and glutathione S-transferase (GST). Preferred peptides are disclosed which comprise a core 7-mer sequence (preferably, a consensus motif) and two or more, preferably at least six, addnl. amino acid residues flanking the core sequence, for a total length of 9, preferably at least 13, amino acid residues and no more than about 45 amino acid residues. Such peptides manifest preferential binding affinities for certain **SH3** domains. The preferred peptides exhibit specific binding affinities for the Src-family of proteins, including Grb2, Yes, Fyn, Lyn, Lck, **Hck**, and Fgr. In vitro and in vivo results are presented which demonstrate the biochem. activity of such peptides. A large no. of proteins not previously suspected of contg. amino acid sequences that bind **SH3** domains are shown to contain such sequences.

REFERENCE COUNT: 1
REFERENCE(S): (1) Knudsen; The EMBO Journal 1995, V14(10), P2191 CA

L3 ANSWER 2 OF 7 CA COPYRIGHT 2001 ACS
ACCESSION NUMBER: 132:75021 CA
TITLE: **SH3** Domains with High Affinity and Engineered Ligand Specificities Targeted to HIV-1 Nef
AUTHOR(S): Hiipakka, Marita; Poikonen, Kari; Saksela, Kalle
CORPORATE SOURCE: Institute of Medical Technology, University of Tampere, Tampere, FIN-33101, Finland
SOURCE: J. Mol. Biol. (1999), 293(5), 1097-1106
CODEN: JMOBAK; ISSN: 0022-2836
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The avid binding of HIV-1 Nef to the Src homol.-3 (**SH3**) domain of **Hck** (KD 250 nM) has been shown to involve an interaction between the RT-loop of **Hck-SH3** and residues in Nef outside of its prototypic polyproline type II (PPII) helix-contg. **SH3**-ligand region. Such distinctive interactions are thought to provide specificity and affinity for other **SH3**/ligand protein complexes as well. Here, we have constructed and successfully displayed on the surface of M13 bacteriophage particles a complex **library** of **SH3** domains, which are derived from **Hck** but carry a random hexapeptide substitution in their RT-loops (termed RRT-**SH3**). Using this strategy we have identified individual RRT-**SH3** domains that can bind to Nef up to 40-fold more avidly than **Hck-SH3**. Some of these high-affinity RRT-**SH3** domains resembled **Hck-SH3** in that they bound much less well to a Nef variant contg. an engineered F90R mutation that interferes with docking of the native **Hck** RT-loop. In addn., we could also select RRT-**SH3** domains with an opposite specificity, which were dependent on the Arg90 residue for strong binding, and bound 100-fold less well to unmodified Nef. These results demonstrate the utility of phage-display in engineering of signaling protein interaction domains, and emphasize the importance of the RT-loop in **SH3** ligand selection, thus suggesting a general strategy for creating **SH3** domains with desired binding properties. (c) 1999 Academic Press.

REFERENCE COUNT: 38
REFERENCE(S): (1) Arold, S; Biochemistry 1998, V37, P14683 CA
(2) Atwell, S; Science 1997, V278, P1125 CAPLUS
(3) Ballinger, M; J Biol Chem 1998, V273, P11675 CA
(4) Cheng, Y; Biochem Pharmacol 1973, V22, P3099 CA
(5) Cohen, G; Cell 1995, V80, P237 CA
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 3 OF 7 CA COPYRIGHT 2001 ACS
ACCESSION NUMBER: 128:125194 CA

TITLE: Exploring the Specificity Pockets of Two Homologous **SH3** Domains Using Structure-Based, Split-Pool Synthesis and Affinity-Based Selection

AUTHOR(S): Kapoor, Tarun M.; Andreotti, Amy Hamilton; Schreiber, Stuart L.

CORPORATE SOURCE: Department of Chemistry and Chemical Biology Howard Hughes Medical Institute, Harvard University, Cambridge, MA, 02138, USA

SOURCE: J. Am. Chem. Soc. (1998), 120(1), 23-29
CODEN: JACSAT; ISSN: 0002-7863

PUBLISHER: American Chemical Society

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Split-pool synthesis was used to prep. large nos. of spatially-sepd. mols. and thereby to investigate the specificity pockets of similar **SH3** domains found in the tyrosine kinases Src and **Hck**. By taking into account the structure of the Src **SH3** domain complexed to a ligand contg. non-peptide-binding elements, the mols. were designed to complement the topog. of the protein's binding pocket. This procedure led to the discovery of ligands having greater affinity and enhanced selectivity for the Src **SH3** domain. It also yielded non-natural ligands that bind selectively to the **Hck SH3** domain. Insights gained from this strategy may facilitate the discovery of mols. useful for evaluating the cellular function of **SH3**-domain-contg. proteins.

L3 ANSWER 4 OF 7 CA COPYRIGHT 2001 ACS

ACCESSION NUMBER: 127:160531 CA

TITLE: Interaction of **SH3** domain of **Hck** tyrosine kinase with cellular proteins containing proline-rich regions: evidence for modulation by unique domain

AUTHOR(S): Gouri, B. S. Vijaya; Swarup, Ghanshyam

CORPORATE SOURCE: Centre for Cellular and Molecular Biology, Hyderabad, 500 007, India

SOURCE: Indian J. Biochem. Biophys. (1997), 34(1&2), 29-39
CODEN: IJBBBQ; ISSN: 0301-1208

PUBLISHER: National Institute of Science Communication

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The **Hck** tyrosine kinase, a member of Src family, is predominantly expressed in myeloid cells. Here, the authors analyzed interaction of cellular proteins with Src homol. 3 (**SH3**) domain of **Hck**. For this purpose they used various GST-**Hck** fusion proteins comprising a part of unique region, complete unique region, and/or complete **SH3** domain of **Hck**, and glutathione S-transferase (GST). When these fusion proteins (or GST), immobilized on glutathione-agarose beads were incubated with [35S] methionine labeled cell exts., multiple proteins which interact specifically with **SH3** domain of **Hck** were detected by SDS-PAGE followed by autoradiog. The **Hck** interacting proteins could also be detected by a tandem blot binding assay in which the blot was incubated with purified fusion protein (or GST) and then the interacting proteins were identified by using antibody against GST. When a part of or complete unique domain was present along with **SH3** domain, the interaction of some specific proteins was reduced several fold. These results raise the possibility of unique domain altering the properties of **SH3** domain, thus modulating or restricting the interaction of **SH3** domain with specific cellular proteins. This modulatory effect of unique domain was localized to 28 amino acids upstream of **SH3** domain. **SH3** interacting proteins were assocd. with serine/threonine and tyrosine kinase activities towards exogenous substrates. Most of the **SH3** binding proteins were

sol. in Triton X-100. Differentiation of promyelocytic leukemia cell line HL-60 into macrophage like cells resulted in appearance of novel **SH3** binding proteins. **Hck** was detected in the eluate of WGA-Sepharose column, suggesting that it interacts with WGA binding glycoprotein (s). A rat spleen cDNA **library** was screened for the **SH3** binding proteins by protein interaction cloning. Sequence anal. of the clones showed the presence of proline rich regions contg. PPXP motifs.

L3 ANSWER 5 OF 7 CA COPYRIGHT 2001 ACS
ACCESSION NUMBER: 123:330857 CA
TITLE: Affinity methods for identifying inhibitors of molecular interactions mediated by **SH3** domains
INVENTOR(S): Rickles, Richard J.; Brugge, Joan S.; Botfield, Martyn C.; Zoller, Mark J.
PATENT ASSIGNEE(S): Ariad Pharmaceuticals, Inc., USA
SOURCE: PCT Int. Appl., 73 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9524419	A1	19950914	WO 1995-US3208	19950313
W: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, ES, FI, GB, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LT, LU, LV, MD, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, US, UZ, VN				
RW: KE, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9521598	A1	19950925	AU 1995-21598	19950313
EP 750630	A1	19970102	EP 1995-914721	19950313
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, NL, PT, SE				
PRIORITY APPLN. INFO.:			US 1994-209835	19940311
			US 1995-369832	19950106
			WO 1995-US3208	19950313

AB Affinity methods for screening peptides that inhibit protein-protein interactions dependent upon **SH3** domains are described for use in the development of therapeutic agents. The method can also be used to identify binding requirements for **SH3**-mediated interactions. The method has identified a no. of unexpected novel, specific, and strongly binding peptides. Methods including screening of a combinatorial phage display **library**, or use of GAL4 fusion proteins with VP16 or **SH3** to regulate expression of a reporter gene are described.

L3 ANSWER 6 OF 7 CA COPYRIGHT 2001 ACS
ACCESSION NUMBER: 123:136465 CA
TITLE: Proline-rich sequences that bind to Src homology 3 domains with individual specificities
AUTHOR(S): Alexandropoulos, Konstantina; Cheng, Genhong; Baltimore, David
CORPORATE SOURCE: Dep. of Biology, Massachusetts Inst. of Technology, Cambridge, MA, 02139, USA
SOURCE: Proc. Natl. Acad. Sci. U. S. A. (1995), 92(8), 3110-4
CODEN: PNASA6; ISSN: 0027-8424
DOCUMENT TYPE: Journal
LANGUAGE: English
AB To study the binding specificity of Src homol. 3 (**SH3**) domains,

we have screened a mouse embryonic expression **library** for peptide fragments that interact with them. Several clones were identified that express fragments of proteins which, through proline-rich binding sites, exhibit differential binding specificity to various **SH3** domains. Src-**SH3**-specific binding uses a sequence of 7 aa of the consensus RPLPXXP, in which the N-terminal arginine is very important. The **SH3** domains of the Src-related kinases Fyn, Lyn, and **Hck** bind to this sequence with the same affinity as that of the Src **SH3**. In contrast, a quite different proline-rich sequence from the Btk protein kinase binds to the Fyn, Lyn, and **Hck** **SH3** domains, but not to the Src **SH3**. Specific binding of the Abl **SH3** requires a longer, more proline-rich sequence but no arginine. One clone that binds to both Src and Abl **SH3** domains through a common site exhibits reversed binding orientation, in that an arginine indispensable for binding to all tested **SH3** domains occurs at the C terminus. Another clone contains overlapping yet distinct Src and Abl **Sh3** binding sites. Binding to the **SH3** domains is mediated by a common PXXP amino acid sequence motif present on all ligands, and specificity comes about from other interactions, often ones involving arginine. The rules governing in vivo usage of particular sites by particular **SH3** domains are not clear, but one binding orientation may be more specific than another.

L3 ANSWER 7 OF 7 CA COPYRIGHT 2001 ACS
ACCESSION NUMBER: 118:142420 CA
TITLE: Identification of rat cDNA encoding **hck** tyrosine kinase from megakaryocytes
AUTHOR(S): Okano, Yukio; Sugimoto, Yoshikazu; Fukuoka, Masami; Matsui, Akira; Nagata, Kohichi; Nozawa, Yoshinori
CORPORATE SOURCE: Sch. Med., Gifu Univ., Gifu, 500, Japan
SOURCE: Biochem. Biophys. Res. Commun. (1991), 181(3), 1137-44
CODEN: BBRCA9; ISSN: 0006-291X
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A rat homolog of **hck** tyrosine kinase cDNA from a rat megakaryocyte **library** contains 1911 nucleotides with an open reading frame encoding 503 amino acids. The rat **hck** had distinct amino acid residues from the mouse homolog, exhibiting 97.6% identity. The sequence contains the SH2 and **SH3** regions that interact with cytoplasmic signaling proteins, the kinase domain including the nucleotide-binding site, and the autophosphorylation site, and the C-terminal Tyr-499 known as a neg. regulator.

=> d his

(FILE 'HOME' ENTERED AT 13:52:37 ON 09 APR 2001)

FILE 'CA' ENTERED AT 13:55:23 ON 09 APR 2001
L1 259 S HCK
L2 54 S L1 AND SH3
L3 7 S L2 AND LIBRARY

=> s l2 not l3

L4 47 L2 NOT L3

=> s l4 and RT loop

33805 RT
72754 LOOP

15 RT LOOP
(RT(W)LOOP)
L5 3 L4 AND RT LOOP

=> d 15 1-3 ibib ab

L5 ANSWER 1 OF 3 CA COPYRIGHT 2001 ACS
ACCESSION NUMBER: 130:1460 CA
TITLE: **RT Loop** Flexibility Enhances the Specificity of Src Family **SH3** Domains for HIV-1 Nef
AUTHOR(S): Arold, Stefan; O'Brien, Ronan; Franken, Peet; Strub, Marie-Paule; Hoh, Francois; Dumas, Christian; Ladbury, John E.
CORPORATE SOURCE: Centre de Biochimie Structurale UMR C9955 CNRS U414 INSERM, Universite Montpellier 1 Faculte de Pharmacie, Montpellier, F34060, Fr.
SOURCE: Biochemistry (1998), 37(42), 14683-14691 CODEN: BICHAW; ISSN: 0006-2960
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Understanding the issue of specificity imposed in the interactions of **SH3** domains has largely been addressed in studies investigating the interaction of proline-rich amino acid sequences derived from potential ligands for these domains. Although the interaction with this motif forms an essential platform in the binding of **SH3** domains, in many cases little specificity is obsd. and the difference in affinity for so-called specific and nonspecific proline-rich sequences is not great. Furthermore, the binding interface between an **SH3** domain and a protein ligand appears to encompass more interactions than are represented by that involving the proline-rich motif. Here we investigate the issue of specificity from the opposite point of view; namely, how does a ligand recognize different **SH3** domains. We present the crystal structure of the unbound **SH3** domain from hemopoietic cell kinase (**Hck**) which is a member of the Src family of tyrosine kinases. This structure reveals that, unlike the structures of other Src kinase **SH3** domains, the **RT loop** region is highly mobile and lacks a network of hydrogen bonds that is elsewhere apparent. The **RT loop** has been shown to form a major part of the binding interface between **SH3** domains and HIV-1 Nef. Thermodn. data, derived from isothermal titrn. calorimetry, for the binding of **Hck SH3** to HIV-1 Nef show that the binding of **Hck** (KD = 1.5 .mu.M) is approx. an order of magnitude tighter than those of other Src family kinases that were investigated (Fyn, Lck, and Src). This increase in affinity is attributed to, among other effects, the inherent flexibility in the **RT loop** which does not require breaking the network of hydrogen bonds to adopt the conformation required for binding.
REFERENCE COUNT: 54
REFERENCE(S):
(1) Alexandropoulos, K; Proc Natl Acad Sci U S A 1995, V92, P3110 CA
(2) Arold, S; Structure 1997, V5, P1361 CA
(3) Briggs, S; J Biol Chem 1997, V272, P17899 CA
(5) Cicchetti, P; Science 1992, V257, P803 CA
(6) Collette, Y; Biol Chem 1996, V271, P6333 CA
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 2 OF 3 CA COPYRIGHT 2001 ACS

ACCESSION NUMBER: 128:11413 CA
TITLE: Identification and localization of slow, natural, cooperative unfolding in the hematopoietic cell kinase **SH3** domain by amide hydrogen exchange and mass spectrometry
AUTHOR(S): Engen, John R.; Smithgall, Thomas E.; Gmeiner, William H.; Smith, David L.
CORPORATE SOURCE: Department of Chemistry, University of Nebraska, Lincoln, NE, 68588-0304, USA
SOURCE: Biochemistry (1997), 36(47), 14384-14391
CODEN: BICHAW; ISSN: 0006-2960
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Protein unfolding on a fast time scale (milliseconds-minutes) has been widely reported, but slower unfolding events (10 min-hours) have received less attention. Here, amide H-exchange (HX) and mass spectrometry (MS) were used to investigate the unfolding dynamics of the **SH3** domain of hematopoietic cell kinase (**Hck**). Anal. of mass spectra after deuterium exchange into intact **Hck SH3** indicated a cooperative unfolding event involving 24-61% of the domain and occurring with a half-life of .apprx.20 min under physiol. conditions. To identify the unfolding region, **SH3** was incubated in D2O and proteolytically fragmented into peptides that were analyzed by mass spectrometry. Correlation of HX rates and isotope patterns revealed cooperative unfolding in several regions, including the C-terminal half of the **RT-loop** and a .beta.-sheet flanking the binding site. Binding of a Pro-rich segment from HIV Nef protein slowed unfolding by a factor of 3. Further anal. yielded a Kd of 25 .mu.M for the Nef peptide. These results demonstrated that an inherent flexibility in the **SH3** domain may assist interconversion of the closed, intramolecularly ligated state and the open, active state of Src family kinases. Furthermore, this type of previously undetectable, slow unfolding process may provide the basis for new mechanisms in which kinetics of local unfolding combines with thermodn. to regulate enzymic activity. The combination of H-exchange and mass spectrometry appears to be the only general method capable of examg. these slow unfolding processes.

L5 ANSWER 3 OF 3 CA
ACCESSION NUMBER: 124:47570 CA
TITLE: A single amino acid in the **SH3** domain of **Hck** determines its high affinity and specificity in binding to HIV-1 Nef protein
AUTHOR(S): Lee, Chi-Hon; Leung, Benjamin; Lemmon, Mark A.; Zheng, Jie; Cowburn, David; Kuriyan, John; Saksela, Kalle
CORPORATE SOURCE: Laboratory Molecular Biophysics, Rockefeller University, New York, NY, 10021, USA
SOURCE: EMBO J. (1995), 14(20), 5006-15
CODEN: EMJODG; ISSN: 0261-4189
DOCUMENT TYPE: Journal
LANGUAGE: English

AB We have examd. the differential binding of **Hck** and Fyn to HIV-1 Nef to elucidate the structural basis of **SH3** binding affinity and specificity. Full-length Nef bound to **Hck SH3** with the highest affinity reported for an **SH3**-mediated interaction (KD 250 nM). In contrast to **Hck**, affinity of the highly homologous Fyn **SH3** for Nef was too weak (KD >20 .mu.M) to

be accurately detd. We show that this distinct specificity lies in a variable loop, the '**RT loop**', positioned close to conserved **SH3** residues implicated in the binding of proline-rich (PxxP) motifs. A mutant Fyn **SH3** with a single amino acid substitution (R96I) in its **RT loop** had an affinity (KD 380 nM) for Nef comparable with that of **Hck SH3**. Based on addnl. mutagenesis studies we propose that the selective recognition of Nef by **Hck SH3** is detd. by hydrophobic interactions involving an isoleucine residue in its **RT loop**. Although Nef contains a PxxP motif which is necessary for the interaction with **Hck SH3**, high affinity binding was only obsd. for intact Nef protein. The binding of a peptide contg. the Nef PxxP motif showed >300-fold weaker affinity for **Hck SH3** than full-length Nef.

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=> d his

     (FILE 'HOME' ENTERED AT 13:52:37 ON 09 APR 2001)

     FILE 'CA' ENTERED AT 13:55:23 ON 09 APR 2001
L1           259 S HCK
L2            54 S L1 AND SH3
L3             7 S L2 AND LIBRARY
L4            47 S L2 NOT L3
L5             3 S L4 AND RT LOOP

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L6            44 L4 NOT L5

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         29506 ALA
        102782 ISO
         28899 HIS
         28899 HIS
         20554 GLU
             0 ASP-ALA-ISO-HIS-HIS-GLU
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L7           0 L6 AND (ASP-ALA-ISO-HIS-HIS-GLU)

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L8           0 L6 AND PLASMIDS

=> s l6 and phagemid

           565 PHAGEMID
L9           0 L6 AND PHAGEMID

=> s l6 and viral

         91313 VIRAL
L10         10 L6 AND VIRAL

=> d l10 1-10 ibib ab


L10  ANSWER 1 OF 10  CA  COPYRIGHT 2001 ACS
ACCESSION NUMBER:        132:264013  CA
TITLE:                   HIV-2 and SIV Nef proteins target different Src
family
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SH3 domains than does HIV-1 Nef because of a triple amino acid substitution

AUTHOR(S): Collette, Yves; Arold, Stefan; Picard, Christophe; Janvier, Katy; Benichou, Serge; Benarous, Richard; Olive, Daniel; Dumas, Christian

CORPORATE SOURCE: U119 INSERM, Universite de la Mediterranee, Marseille, 13009, Fr.

SOURCE: J. Biol. Chem. (2000), 275(6), 4171-4176
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The nef gene is required for optimal **viral** spread of human and simian immunodeficiency viruses. However, the mol. mechanisms underlying the action of the Nef proteins may not be identical for all **viral** families. Here the authors investigate the interaction between the Nef protein of human and simian immunodeficiency viruses and **SH3** domains from Src family kinases. Using the yeast 2-hybrid system and immunoblotting they show that, in contrast to HIV-1 Nef, SIV and HIV-2 Nef poorly interact with **Hck SH3** but bind to Src and Fyn **SH3** domains. The mol. basis of these differences in **SH3** targeting was revealed by sequence anal. and homol. modeling of the putative **SH3**-Nef structures. Three amino acids (Trp-113, Thr-117, and Gln-118) that localize in a "hydrophobic pocket" implicated in **SH3** binding of HIV-1 Nef, are systematically substituted in SIV/HIV-2 alleles (by Tyr, Glu, and Glu, resp.). The authors demonstrate that site-directed mutagenesis of these residues in SIVmac239 Nef suffices to restore **Hck SH3** binding and co-immunopptn. with full-length **Hck** from transfected cells. These findings thus identify fundamental mechanistic differences in targeting of Src family kinases by HIV and SIV Nef. The herein described mechanism of **SH3** selection by Nef via a "pocket" proximal to the canonical proline-rich motif may be a common feature for **SH3** recognition by their natural ligands.

REFERENCE COUNT: 33
REFERENCE(S): (1) Arold, S; Biochemistry 1998, V37, P14683 CA
(2) Arold, S; Structure 1997, V5, P1361 CA
(3) Baur, A; Immunity 1997, V6, P283 CA
(4) Benichou, S; J Biol Chem 1994, V269, P30073 CA
(5) Briggs, S; J Biol Chem 1997, V272, P17899 CA
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L10 ANSWER 2 OF 10 CA

ACCESSION NUMBER: 132:133984 CA

TITLE: Affinity of Src Family Kinase **SH3** Domains for HIV Nef in Vitro Does Not Predict Kinase Activation by Nef in Vivo

AUTHOR(S): Briggs, Scott D.; Lerner, Edwina C.; Smithgall, Thomas E.

CORPORATE SOURCE: Eppley Institute for Research in Cancer and Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, NE, 68198, USA

SOURCE: Biochemistry (2000), 39(3), 489-495
CODEN: BICHAW; ISSN: 0006-2960
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Nef is an HIV accessory protein required for high-titer **viral** replication and AIDS progression. Previous studies have shown that the

SH3 domains of **Hck** and Lyn bind to Nef via proline-rich sequences in vitro, identifying these Src-related kinases as potential targets for Nef in vivo. Assocn. of Nef with **Hck** causes displacement of the intramol. interaction between the **SH3** domain and the SH2-kinase linker, leading to kinase activation both in vitro and in vivo. In this study, we investigated whether interaction with Nef induces activation of other Src family kinases (Lyn, Fyn, Src, and Lck) following coexpression with Nef in Rat-2 fibroblasts. Coexpression with Nef induced **Hck** kinase activation and fibroblast transformation, consistent with previous results. In contrast, coexpression of Nef with Lyn was without effect, despite equiv. binding of Nef to full-length Lyn and **Hck**. Furthermore, Nef was found to suppress the kinase and transforming activities of Fyn, the **SH3** domain of which exhibits low affinity for Nef. Coexpression with Nef did not alter c-Src or Lck tyrosine kinase or transforming activity in this system. Differential modulation of Src family members by Nef may produce unique downstream signals depending on the profile of Src kinases expressed in a given cell type.

REFERENCE COUNT: 44
REFERENCE(S):
(1) Arold, S; Biochemistry 1998, V37, P14683 CA
(2) Arold, S; Structure 1997, V5, P1361 CA
(3) Bahnson, A; J Virol Methods 1995, V54, P131 CA
(4) Briggs, S; J Biol Chem 1995, V270, P14718 CA
(5) Briggs, S; J Biol Chem 1997, V272, P17899 CA
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L10 ANSWER 3 OF 10 CA

ACCESSION NUMBER: 132:92210 CA

TITLE: The HIV Nef protein alters Ca2+ signaling in myelomonocytic cells through **SH3**-mediated protein-protein interactions

AUTHOR(S): Foti, Michelangelo; Cartier, Laetitia; Piguet, Vincent; Lew, Daniel P.; Carpentier, Jean-Louis; Trono, Didier; Krause, Karl-Heinz

CORPORATE SOURCE: Department of Morphology, University of Geneva, Geneva, CH-1225, Switz.

SOURCE: J. Biol. Chem. (1999), 274(49), 34765-34772
CODEN: JBCHA3; ISSN: 0021-9258

PUBLISHER: American Society for Biochemistry and Molecular Biology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Human immunodeficiency virus Nef plays an important role in AIDS pathogenesis. In addn. to the well known down-regulation of cell surface receptors (CD4, MHCI), Nef is able to alter cellular signaling. Of particular interest for this study is the ability of Nef to bind with a very high affinity to **SH3** domains of myelomonocyte-specific protein-tyrosine kinases of the Src family (Src-like PTK). The authors have therefore investigated Ca2+ signaling in HL60 cells retrovirally transduced with wild type Nef or with a Nef mutant deficient in the **SH3**-interacting proline-rich motif (Nef(PXXP)4-). In differentiated HL60 cells, Nef markedly altered cellular Ca2+ signaling; the amt. of intracellularly stored Ca2+ was increased, and as a consequence, store-operated Ca2+-influx was decreased. This effect was not obsd. in undifferentiated HL60 cells or in CEM T-lymphocytes and correlated with the differentiation-induced up-regulation of Src-like PTK.

The Nef effect on Ca2+ signaling depended entirely on the integrity of its PXXP motif. The Src-like PTK p56/59hck co-immunopptd. with both Nef and with the inositol 1,4,5-trisphosphate receptor, providing a possible mechanistic link between the **viral** protein and intracellular Ca2+ stores of the host cell. Collectively, the results demonstrate that the human immunodeficiency virus 1 Nef protein manipulates intracellular Ca2+ stores through **SH3**-mediated interactions in myelomonocytic

cells.
REFERENCE COUNT: 65
REFERENCE(S): (1) Aiken, C; Cell 1994, V76, P853 CA
(2) Aiken, C; J Virol 1995, V69, P5048 CA
(3) Aiken, C; Virology 1996, V217, P293 CA
(4) Alexander, L; J Virol 1997, V71, P6094 CA
(5) Baur, A; Immunity 1997, V6, P283 CA
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L10 ANSWER 4 OF 10 CA COPYRIGHT 2001 ACS
ACCESSION NUMBER: 132:48903 CA
TITLE: The conserved core of human immunodeficiency virus type 1 Nef is essential for association with Lck and for enhanced **viral** replication in T-lymphocytes
AUTHOR(S): Cheng, Hua; Hoxie, James; Parks, Wade P.
CORPORATE SOURCE: Department of Microbiology and Pediatrics, New York University School of Medicine, New York, NY, 10016, USA
SOURCE: Virology (1999), 264(1), 5-15
CODEN: VIRLAX; ISSN: 0042-6822
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The Nef protein of the primate lentiviruses, including human immunodeficiency virus type 1 (HIV-1) and simian immunodeficiency virus (SIV), is a myristylated protein assocd. with increased **viral** replication and enhanced pathogenicity. Both the potentiation of T-lymphocyte activation and the enhanced serine-phosphorylation of HIV-1 capsid by Nef correlate with increased **viral** replication. The authors report the functional interactions of the Nef proteins with Src kinases. The Nef proteins from HIV-1 and SIV bind to Lck as well as **Hck**, Lyn, and Fyn. The **SH3** and SH2 domains of Lck are sufficient for copptn. with non-tyrosine-phosphorylated Nef proteins. The conserved core region of HIV-1 Nef is essential for the interaction with Lck and is also important for enhanced HIV-1 replication in T-lymphocytes.
In addn., the authors show that SIV and HIV-1 Nef proteins are differentially tyrosine-phosphorylated. The kinase-active Lck tyrosine-phosphorylates SIVmac239 Nef but does not phosphorylate HIV-1 Nef. These data suggest that the assocn. of Nef and Lck is central to the enhanced **viral** replication of HIV-1 and SIV in T-lymphocytes.
(c) 1999 Academic Press.
REFERENCE COUNT: 53
REFERENCE(S): (1) Aiken, C; J Virol 1995, V69, P5048 CA
(2) Alexander, L; J Virol 1997, V71, P6094 CA
(3) Aronheim, A; Cell 1994, V78, P949 CA
(4) Baur, A; Immunity 1994, V1, P373 CA
(5) Baur, A; Immunity 1997, V6, P283 CA
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L10 ANSWER 5 OF 10 CA COPYRIGHT 2001 ACS
ACCESSION NUMBER: 131:167530 CA
TITLE: Simian immunodeficiency virus and human immunodeficiency virus type 1 Nef proteins show distinct patterns and mechanisms of Src kinase activation
AUTHOR(S): Greenway, Alison L.; Dutartre, Helene; Allen, Kelly; McPhee, Dale A.; Olive, Daniel; Collette, Yves
CORPORATE SOURCE: AIDS Cellular Biology Unit, Macfarlane Burnet Center for Medical Research, Fairfield, 3078, Australia
SOURCE: J. Virol. (1999), 73(7), 6152-6158
CODEN: JOVIAM; ISSN: 0022-538X

PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The nef gene from human and simian immunodeficiency viruses (HIV and SIV) regulates cell function and **viral** replication, possibly through binding of the nef product to cellular proteins, including Src family tyrosine kinases. It is shown here that the Nef protein encoded by SIVmac239 interacts with and also activates the human Src kinases Lck and **Hck**. This is in direct contrast to the inhibitory effect of HIV type 1 (HIV-1) Nef on Lck catalytic activity. Unexpectedly, however, the interaction of SIV Nef with human Lck or **Hck** is not mediated via its consensus proline motif, which is known to mediate HIV-1 Nef binding to Src homol. 3 (**SH3**) domains, and various exptl. analyses failed to show significant interaction of SIV Nef with the **SH3** domain of either kinase. Instead, SIV Nef can bind Lck and **Hck** SH2 domains, and its N-terminal 50 amino acid residues are sufficient for Src kinase binding and activation. These results provide evidence for multiple mechanisms by which Nef binds to and regulates Src kinases.

REFERENCE COUNT: 42
REFERENCE(S):
(1) Aiken, C; J Virol 1995, V69, P5048 CA
(2) Arold, S; Structure 1997, V5, P1361 CA
(3) Azad, A; J Gen Virol 1994, V75, P651 CA
(4) Baur, A; Immunity 1997, V6, P283 CA
(5) Benson, R; J Exp Med 1993, V177, P1561 CA
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L10 ANSWER 6 OF 10 CA COPYRIGHT 2001 ACS
ACCESSION NUMBER: 131:126909 CA
TITLE: Herpesvirus ateles gene product Tio interacts with nonreceptor protein tyrosine kinases
AUTHOR(S): Albrecht, Jens-Christian; Friedrich, Ute; Kardinal, Christian; Koehn, Jadranka; Fleckenstein, Bernhard; Feller, Stephan M.; Biesinger, Brigitte
CORPORATE SOURCE: Institut fur Klinische und Molekulare Virologie, Universitat Erlangen-Nurnberg, Erlangen, 91054, Germany
SOURCE: J. Virol. (1999), 73(6), 4631-4639
CODEN: JOVIAM; ISSN: 0022-538X
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Herpesvirus ateles is a gamma-2-herpesvirus which naturally infects spider monkeys (Ateles spp.) and causes malignant lymphoproliferative disorders in various other New World primates. The genomic sequence of herpesvirus ateles strain 73 revealed a close relationship to herpesvirus saimiri, with a high degree of variability within the left terminus of the coding region. A spliced mRNA transcribed from this region was detected in New World monkey T-cell lines transformed by herpesvirus ateles in vitro or derived from T cells of infected Saguinus oedipus. The encoded **viral** protein, termed Tio, shows restricted homol. to the oncoprotein StpC and to the tyrosine kinase-interacting protein Tip, two gene products responsible for the T-cell-transforming and oncogenic phenotype of herpesvirus saimiri group C strains. Tio was detectable in lysates of the transformed T lymphocytes. Dimer formation was obsd. after expression of recombinant Tio. After cotransfection, Tio was phosphorylated in vivo by the protein tyrosine kinases Lck and Src and less efficiently by Fyn. Stable complexes of these Src family kinases with the **viral** protein were detected in lysates of the transfected cells. Binding analyses indicated a direct interaction of Tio with the **SH3** domains of Lyn, **Hck**, Lck, Src, Fyn, and Yes. In addn., tyrosine-phosphorylated Tio bound to the SH2 domains of Lck, Src, or Fyn. Thus, herpesvirus ateles-encoded Tio may contribute to

viral T-cell transformation by influencing the function of Src family kinases.

REFERENCE COUNT: 79
REFERENCE(S):
(1) Albrecht, J; J Virol 1992, V66, P5047 CA
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(6) Biesinger, B; Virology 1990, V176, P505 CA
(7) Bolen, J; Cell 1984, V38, P767 CA
(8) Caldwell, R; Immunity 1998, V9, P405 CA
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L10 ANSWER 7 OF 10 CA COPYRIGHT 2001 ACS
ACCESSION NUMBER: 131:113573 CA
TITLE: The cellular kinase binding motifs (PxxP and RR) in human immunodeficiency virus type 1 Nef protein are dispensable for producer-cell-dependent enhancement of **viral** entry
AUTHOR(S): Tokunaga, Kenzo; Ikuta, Kazuyoshi; Adachi, Akio; Matsuda, Michiyuki; Kurata, Takeshi; Kojima, Asato
CORPORATE SOURCE: Department of Pathology, National Institute of Infectious Diseases, Tokyo, 162-8640, Japan
SOURCE: Virology (1999), 257(2), 285-289
CODEN: VIRLAX; ISSN: 0042-6822
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB We have recently demonstrated that human immunodeficiency virus type 1 (HIV-1) Nef is required for enhancing **viral** infectivity by increasing the efficiency of **viral** entry in a producer-cell-dependent manner, suggesting the possible involvement of a cellular factor(s) in the enhancement of **viral** entry. Moreover, it has been reported that a proline-rich (PxxP) motif and an Arg-Arg (RR) motif in HIV-1 Nef bind to the **SH3** domain of the Src-family tyrosine kinase **Hck** and to a serine/threonine kinase, resp. To address whether these cellular kinase binding motifs, PxxP and RR, could be involved in virus producer-cell-dependent enhancement of **viral** entry, we constructed two nef mutant proviral clones in which these motifs were mutated. The results show that the HIV-1 Nef PxxP motif, which significantly influenced **viral** infectivity, and the RR motif, which modestly affected **viral** infectivity, were both dispensable for enhanced **viral** entry, thus suggesting that another interaction of Nef with a cellular factor(s) is involved in the efficiency of **viral** entry. (c) 1999 Academic Press.

REFERENCE COUNT: 31
REFERENCE(S):
(1) Adachi, A; Arch Virol 1991, V117, P45 CA
(3) Aiken, C; J Virol 1995, V69, P5048 CA
(4) Aiken, C; Virology 1996, V217, P293 CA
(5) Aldrovandi, G; J Virol 1998, V72, P7032 CA
(6) Chowers, M; J Virol 1994, V68, P2906 CA
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L10 ANSWER 8 OF 10 CA COPYRIGHT 2001 ACS
ACCESSION NUMBER: 130:65131 CA
TITLE: **SH3**-domain binding function of HIV-1 Nef is required for association with a PAK-related kinase
AUTHOR(S): Manninen, Aki; Hiipakka, Marita; Vihinen, Mauno; Lu, Wange; Mayer, Bruce J.; Saksela, Kalle
CORPORATE SOURCE: Institute of Medical Technology, University of Tampere, Tampere, FIN-33101, Finland
SOURCE: Virology (1998), 250(2), 273-282
CODEN: VIRLAX; ISSN: 0042-6822
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal

LANGUAGE: English

AB HIV-1 Nef has previously been shown to bind to Src homol.-3 (**SH3**) domains of a subset of Src family tyrosine kinases. In addn., Nef has been reported to coppt. with a serine/threonine kinase activity termed NAK (for Nef-assocd. kinase). The identity of NAK remains uncertain, but it has been suggested to represent a novel member of the p21-activated kinase (PAK) family. The authors report here that NAK autophosphorylation is increased not only by an activated form of the p21-family GTPase cdc42 but also by a plasma membrane-targeted fragment of the adapter protein Nck, thus providing further evidence that NAK is related to PAKs. A detailed structure-based mutational anal. of Nef revealed that all amino acid changes that inhibited the Nef/**Hck-SH3** interaction, as measured by surface-plasmon resonance, also abolished copptn. of NAK. As PAK family proteins do not contain **SH3** domains, these observations are best explained by a protein complex in which Nef, NAK, and an **SH3**-protein all contact each other. In addn., a no. of conserved amino acids in Nef that are not involved in **SH3** binding were also crucial for assocn. with NAK. Mol. modeling suggests that these residues are involved in formation of an adjacent binding surface for NAK or another crit. component of the NAK/Nef complex. (c) 1998 Academic Press.

REFERENCE COUNT: 30

REFERENCE(S):
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(2) Briggs, S; J Biol Chem 1997, V272, P17899 CA
(3) Collette, Y; J Biol Chem 1996, V271, P6333 CA
(4) Feng, S; Science 1994, V266, P1241 CA
(5) Goldsmith, M; Nef J Virol 1995, V69, P4112 CA
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L10 ANSWER 9 OF 10 CA COPYRIGHT 2001 ACS

ACCESSION NUMBER: 129:244012 CA

TITLE: The human immunodeficiency virus type 1 NEF protein binds the Src-related tyrosine kinase Lck SH2 domain through a novel phosphotyrosine independent mechanism

AUTHOR(S): Dutartre, Helene; Harris, Mark; Olive, Daniel; Collette, Yves

CORPORATE SOURCE: Unite 119 Instituto National de la Sante et de la Recherche Medicale, Marseille, 13009, Fr.

SOURCE: Virology (1998), 247(2), 200-211
CODEN: VIRLAX; ISSN: 0042-6822

PUBLISHER: Academic Press

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Primate lentiviruses encode for a unique nef gene with an essential function in both **viral** replication and pathogenicity in the host. The mol. basis for this function remains however poorly defined. Several Nef-binding cellular proteins are thought to be instrumental in its function. Indeed, Nef contains a proline-rich motif implicated in the binding to the Src-like tyrosine kinase **Hck** and also to a Ser/Thr kinase of mol. wt. 62 kDa. The disruption of this motif affects the binding to both these kinases as well as **viral** replication. Whereas **Hck** is expressed in the myeloid lineage and hence may account for the nef function in infected monocytes, the authors and others have reported previously that Nef also interacts with the T-lymphocyte Src-kinase Lck, leading to specific cell signaling impairment. This interaction occurs through the binding of Nef to both Lck SH2 and **SH3** domains. Both the proline motif and phosphorylation of Nef on tyrosine residue were proposed to account for these interactions. Here, the authors investigate the mechanism of Lck SH2 binding by HIV-1 Nef. Using recombinant fusion proteins to ppt. lysates, they show that although

SH2 binding is dependent on phosphorylation events, it occurs in a tyrosine independent manner because it requires neither tyrosine residues in Nef nor the phosphotyrosine binding pocket from the Lck SH2 domain, hence suggesting a role for a phosphoserine or a phosphothreonine residue.

Further, the authors show that **Hck** SH2 does not interact with Nef, indicating that **Hck SH3** binding is sufficient for Nef binding, whereas Lck SH2 cooperates together with **SH3** to allow Nef binding to a level similar to **Hck SH3**. Together, the authors' results establish different mechanisms for **Hck** and Lck binding by HIV-1 Nef protein, and identify a novel mechanism for Src-like tyrosine kinase targeting by a **viral** protein. (c) 1998 Academic Press.

L10 ANSWER 10 OF 10 CA COPYRIGHT 2001 ACS
ACCESSION NUMBER: 122:158441 CA
TITLE: Proline-rich (PxxP) motifs in HIV-1 Nef bind to **SH3** domains of a subset of Src kinases and are required for the enhanced growth of Nef+ viruses but not for down-regulation of CD4
AUTHOR(S): Saksela, Kalle; Cheng, Genhong; Baltimore, David
CORPORATE SOURCE: Rockefeller Univ., New York, NY, 10021, USA
SOURCE: EMBO J. (1995), 14(3), 484-91
CODEN: EMJODG; ISSN: 0261-4189
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Human immunodeficiency virus (HIV) and simian immunodeficiency virus Nef proteins contain a conserved motif with the minimal consensus (PxxP) site for Src homol. region 3 (**SH3**)-mediated protein-protein interactions. Nef PxxP motifs show specific binding to biotinylated **SH3** domains of **Hck** and Lyn, but not to those of other tested Src family kinases or less related proteins. A unique cooperative role of a distant proline is also obsd. Endogenous **Hck** of monocytic U937 cells can be specifically pptd. by matrix-bound HIV-1 Nef, but not by mutant protein lacking PxxP. Intact Nef PxxP motifs are dispensable for Nef-induced CD4 down-regulation, but are required for the higher in vitro replicative potential of Nef+ viruses. Thus, CD4 down-regulation and promotion of **viral** growth are two distinct functions of Nef, and the latter is mediated via **SH3** binding.

=> d his

(FILE 'HOME' ENTERED AT 13:52:37 ON 09 APR 2001)

FILE 'CA' ENTERED AT 13:55:23 ON 09 APR 2001
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L2 54 S L1 AND SH3
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L4 47 S L2 NOT L3
L5 3 S L4 AND RT LOOP
L6 44 S L4 NOT L5
L7 0 S L6 AND (ASP-ALA-ISO-HIS-HIS-GLU)
L8 0 S L6 AND PLASMIDS
L9 0 S L6 AND PHAGEMID
L10 10 S L6 AND VIRAL

=> s l6 not l10

L11 34 L6 NOT L10

=> d l11 1-34 ab ti

L11 ANSWER 1 OF 34 CA COPYRIGHT 2001 ACS
AB A review with 54 refs. including protocols. The combined technique of hydrogen exchange/mass spectrometry may be used to learn more about proteins aside from their mol. mass. Zhang and Smith have developed a method of deuterium labeling followed by pepsin digestion to localize exchange to short fragments of the protein backbone. The general method is described in detail. This approach has been used to investigate many structural features of proteins including structural heterogeneity in oxidized cytochrome c; the effects of mutagenesis on ferredoxin, ferrocytochrome c2 and cytochrome c553; small mol. (NADPH) binding-induced conformational changes in diaminopimelate dehydrogenase and dihydrodipicolinate reductase and changes in protein structure caused by rhodopsin binding to arrestin; exchange rates in portions of apo- and holo-myoglobin; slow protein unfolding in **Hck SH3** and the effects of peptide binding; and protein kinase activation in wild-type and mutant MAP kinase kinase-1.
TI Investigating the higher order structure of proteins: hydrogen exchange, proteolytic fragmentation, and mass spectrometry

L11 ANSWER 2 OF 34 CA COPYRIGHT 2001 ACS
AB The Src homol. 2 (SH2) and Src homol. 3 (**SH3**) domains of Src family kinases are involved in substrate recognition in vivo. Many cellular substrates of Src kinases contain a large no. of potential phosphorylation sites, and the SH2 and **SH3** domains of Src are known to be required for phosphorylation of these substrates. In principle, Src could phosphorylate these substrates by either a processive mechanism, in which the enzyme remains bound to the peptide substrate during multiple phosphorylation events, or a nonprocessive (distributive) mechanism, where each phosphorylation requires a sep. binding interaction between enzyme and substrate. Here we use a synthetic peptide system to demonstrate that **Hck**, a Src family kinase, can phosphorylate substrates contg. an SH2 domain ligand by a processive mechanism. **Hck** catalyzes the phosphorylation of these sites in a defined order. Furthermore, we show that addn. of an **SH3** domain to a peptide can enhance its phosphorylation both by activating **Hck** and by increasing the affinity of the substrate. On the basis of our observations on the role of the SH2 and **SH3** domains in substrate recognition, we present a model for substrate targeting in vivo.
TI A Peptide Model System for Processive Phosphorylation by Src Family Kinases

L11 ANSWER 3 OF 34 CA COPYRIGHT 2001 ACS
AB Background: Src homol. 3 (**SH3**) domains bind sequences bearing the consensus motif PxxP (where P is proline and x is any amino acid), wherein domain specificity is mediated largely by sequences flanking the PxxP core. This specificity is limited, however, as most **SH3** domains show high ligand cross-reactivity. We have recently shown that diverse N-substituted residues (peptoids) can replace the prolines in the PxxP motif, yielding a new source of ligand specificity. Results: We have tested the effects of combining multiple peptoid substitutions with specific flanking sequences on ligand affinity and specificity. We show that by varying these different elements, a ligand can be selectively tuned to target a single **SH3** domain in a test set. In addn., we show that by making multiple peptoid substitutions, high-affinity ligands can be generated that completely lack the canonical PxxP motif. The resulting ligands can potently disrupt natural **SH3**-mediated interactions. Conclusions: Peptide-peptoid hybrid scaffolds yield **SH3** ligands with markedly improved domain selectivity, overcoming one of the principal challenges in designing inhibitors against these domains. These compds. represent important leads in the search for orthogonal inhibitors of **SH3** domains, and can serve as tools for

the dissection of complex signaling pathways.
TI Improving **SH3** domain ligand selectivity using a non-natural scaffold

L11 ANSWER 4 OF 34 CA COPYRIGHT 2001 ACS
AB Bcr-Abl is the constitutively active protein-tyrosine kinase expressed as a result of the Philadelphia translocation in chronic myelogenous leukemia. Bcr-Abl is coupled to many of the same signaling pathways normally regulated by hematopoietic cytokines. Recent work shows that **Hck**, a member of the Src tyrosine kinase family with myeloid-restricted expression, assocs. with and is activated by Bcr-Abl. Here we investigated the mechanism of **Hck** interaction with Bcr-Abl and the requirement for **Hck** activation in Bcr-Abl transformation signaling. Binding studies demonstrated that the **Hck SH3** and SH2 domains are sufficient for interaction with Bcr-Abl in vitro. **Hck** binding localizes to the Abl SH2, **SH3**, and kinase domains as well as the distal portion of the C-terminal tail. To address the requirement for endogenous Src family kinase activation in Bcr-Abl signaling, a kinase-defective mutant of **Hck** was stably expressed in the cytokine-dependent myeloid leukemia cell line DAGM. Kinase-defective **Hck** dramatically suppressed Bcr-Abl-induced outgrowth of these cells in the absence of cytokine compared with a control cell line expressing .beta.-galactosidase. In contrast, kinase-defective **Hck** did not affect cell proliferation in response to interleukin-3, suggesting that the effect is specific for Bcr-Abl. These data show that **Hck** interacts with Bcr-Abl through a complex mechanism involving kinase-dependent and -independent components and that interaction with **Hck** or other Src family members is essential for transformation signaling by Bcr-Abl.
TI Transformation of myeloid leukemia cells to cytokine independence by Bcr-Abl is suppressed by kinase-defective **Hck**

L11 ANSWER 5 OF 34 CA COPYRIGHT 2001 ACS
AB Src family tyrosine kinases have previously been proposed to mediate some of the biol. effects of lipopolysaccharide on macrophages. Accordingly, the authors have sought to identify substrates of Src family kinases in lipopolysaccharide-stimulated macrophages. Stimulation of Bac1.2F5 macrophage cells with lipopolysaccharide was found to induce gradual and persistent tyrosine phosphorylation of Cbl in an Src family kinase-dependent manner. Immunopptn. expts. revealed that Cbl assocs. with **Hck** in Bac1.2F5 cells, while expression of an activated form of **Hck** in Bac1.2F5 cells induces tyrosine phosphorylation of Cbl in the absence of lipopolysaccharide stimulation. The Src homol. 3
domain of **Hck** can directly bind Cbl, and this interaction is important for phosphorylation of Cbl. Assocn. of the p85 subunit of phosphatidylinositol (PI) 3-kinase with Cbl is enhanced following lipopolysaccharide stimulation of Bac1.2F5 cells, and transient expression
expts. indicate that phosphorylation of Cbl by **Hck** can facilitate the assocn. of p85 with Cbl. Lipopolysaccharide treatment also
stimulates the partial translocation of **Hck** to the cytoskeleton of Bac1.2F5 cells. Notably, lipopolysaccharide enhances the adherence of Bac1.2F5 cells, an effect that is dependent on the activity of Src family kinases and PI 3-kinase. Thus, the authors postulate that **Hck** enhances the adherence of lipopolysaccharide-stimulated macrophages, at least in part, via Cbl and PI 3-kinase.
TI **Hck** enhances the adherence of lipopolysaccharide-stimulated macrophages via Cbl and phosphatidylinositol 3-kinase

L11 ANSWER 6 OF 34 CA COPYRIGHT 2001 ACS
AB Although most L-type calcium channel .alpha.1C subunits isolated from heart or brain are .apprx.190-kDa proteins that lack .apprx.50 kDa of the

C terminus, the C-terminal domain is present in intact cells. To test the hypothesis that the C terminus is processed but remains functionally assocd. with the channels, expressed, full-length .alpha.1C subunits were cleaved in vitro by chymotrypsin to generate a 190-kDa C-terminal truncated protein and C-terminal fragments of 30-56 kDa. These hydrophilic C-terminal fragments remained membrane-assocd. A C-terminal proline-rich domain (PRD) was identified as the mediator of membrane assocn. The .alpha.1C PRD bound to **SH3** domains in Src, Lyn, **Hck**, and the channel .beta.2 subunit. Mutant .alpha.1C subunits lacking either .apprx.50 kDa of the C terminus or the PRD produced increased barium currents through the channels, demonstrating that these domains participate in the previously described inhibition of channel function by the C terminus.

TI Proteolytic processing of the C terminus of the .alpha.1C subunit of L-type calcium channels and the role of a proline-rich domain in membrane tethering of proteolytic fragments

L11 ANSWER 7 OF 34 CA COPYRIGHT 2001 ACS

AB Mol. dynamics calcns. provide a method by which the dynamic properties of mols. can be explored over timescales and at a level of detail that cannot be obtained exptl. from NMR or x-ray analyses. Recent work has indicated that the accuracy of these simulations is high, as measured by the correspondence of parameters extd. from these calcns. to those detd. through exptl. means. Here, we investigate the dynamic behavior of the Src homol. 3 (**SH3**) domain of hematopoietic cell kinase (**Hck**) via 15N backbone relaxation NMR studies and a set of four independent 4 ns solvated mol. dynamics calcns. We also find that mol. dynamics simulations accurately reproduce fast motion dynamics as estd. from generalized order parameter (S2) anal. for regions of the protein that have exptl. well-defined coordinates (i.e., stable secondary structural elements). However, for regions where the coordinates are not well defined, as indicated by high local root-mean-square deviations among NMR-detd. structural family members or high B-factors/low electron d. in x-ray crystallog. detd. structures, the parameters calcd. from a short to moderate length (less than 5-10 ns) mol. dynamics trajectory are dependent on the particular coordinates chosen as a starting point for the simulation.

TI Dynamics of the **Hck-SH3** domain: comparison of experiment with multiple molecular dynamics simulations

L11 ANSWER 8 OF 34 CA COPYRIGHT 2001 ACS

AB The Src family tyrosine kinase **Hck** possesses two phosphorylation sites, Tyr527 and Tyr416, that affect the catalytic activity in opposite ways. When phosphorylated, Tyr527 and residues C-terminal to it are involved in an inhibitory intramol. interaction with the SH2 domain. However, this sequence does not conform to the sequence of the high affinity SH2 ligand, pYEEI. We mutated this sequence to YEEI and show that this mutant form of **Hck** cannot be activated by exogenous SH2 ligands. The **SH3** domain of **Hck** is also involved in an inhibitory interaction with the catalytic domain. The **SH3** ligand Nef binds to and activates YEEI-**Hck** mutant in a similar manner to wild-type **Hck**, indicating that disrupting the **SH3** interaction overrides the strengthened SH2 interaction. The other phosphorylation site, Tyr416, is the autophosphorylation site in the activation loop. Phosphorylation of Tyr416 is required for **Hck** activation. We mutated this residue to alanine and characterized its catalytic activity. The Y416A mutant shows a higher Km value for peptide and a lower Vmax than autophosphorylated wild-type **Hck**. We also present evidence for cross-talk between the activation loop and the intramol. binding of the SH2 and **SH3** domains.

TI Reciprocal regulation of **Hck** activity by phosphorylation of Tyr527 and Tyr416. Effect of introducing a high affinity intramolecular SH2 ligand

L11 ANSWER 9 OF 34 CA COPYRIGHT 2001 ACS
AB Biochem. and structural studies of Src and related kinases demonstrate that two intramol. interactions suppress kinase activity. These interactions involve binding of the SH2 domain to a phosphotyrosine residue in the C-terminal tail and assocn. of the **SH3** domain with a polyproline type II helix formed by amino acids linking the SH2 and kinase domains. Recent studies have shown that high affinity interaction of the **SH3** domain of **Hck** with the human immunodeficiency virus type I Nef protein activates **Hck** tyrosine kinase and biol. activities, suggesting a mechanism that involves disruption of the **SH3**-linker interaction. To test the role of this interaction in the regulation of **Hck** kinase activity in living cells, we substituted alanines for prolines 225 and 228 in the linker region and obsd. that the resulting mutant (**Hck**-2PA) demonstrated strong transforming activity in a Rat-2 fibroblast focus-forming assay. **Hck**-2PA also exhibited elevated tyrosine kinase activity in terms of autophosphorylation, endogenous substrate phosphorylation, and in an in vitro kinase assay. The transforming and kinase activities of **Hck**-2PA were remarkably similar to those obsd. with a **Hck** mutant activated by Phe substitution of the conserved tail Tyr residue and with wild-type **Hck** following co-expression with human immunodeficiency virus Nef. Introduction of the 2PA and tail mutations into a single **Hck** expression construct did not increase kinase or transforming activity relative to the individual mutations. These data provide new evidence that **SH3** -linker interaction may represent the dominant mechanism controlling **Hck** tyrosine kinase activity in vivo.
TI SH2-kinase linker mutations release **Hck** tyrosine kinase and transforming activities in rat-2 fibroblasts

L11 ANSWER 10 OF 34 CA COPYRIGHT 2001 ACS
AB The crystal structure of the autoinhibited form of **Hck** has been detd. at 2.0 .ANG. resoln., in complex with a specific pyrazolo pyrimidine-type inhibitor, PP1. The activation segment, a key regulatory component of the catalytic domain, is unphosphorylated and is visualized in its entirety. Tyr-416, the site of activating autophosphorylation in the Src family kinases, is positioned such that access to the catalytic machinery is blocked. PP1 is bound at the ATP-binding site of the kinase, and a methylphenyl group on PP1 is inserted into an adjacent hydrophobic pocket. The enlargement of this pocket in autoinhibited Src kinases suggests a route toward the development of inhibitors that are specific for the inactive forms of these proteins.
TI Crystal structure of **HcK** in complex with a Src family-selective tyrosine kinase inhibitor

L11 ANSWER 11 OF 34 CA COPYRIGHT 2001 ACS
AB Protein dynamics play an important role in protein function and regulation of enzymic activity. To det. how addnl. interactions with surrounding structure affects local protein dynamics, we have used hydrogen exchange and mass spectrometry to investigate the SH2 and **SH3** domains of the protein tyrosine kinase **Hck**. Exchange rates of isolated **Hck SH3** and SH2 domains were compared with rates for the same domains when part of a larger SH(3+2) construct. Increased deuterium incorporation was obsd. for the **SH3** domain in the joint construct, particularly near the SH2 interface and the short sequence that connects **SH3** to SH2, implying greater flexibility of **SH3**

when it is part of SH(3+2). Slow cooperative unfolding of the **SH3** domain occurred at the same rate in isolated **SH3** as in the SH(3+2) construct, suggesting a functional significance for this unfolding. The SH2 domain displayed relatively smaller changes in flexibility when part of the SH(3+2) construct. These results suggest that the domains influence each other. Further, our results imply a link between functional regulation and structural dynamics of **SH3** and SH2 domains. (c) 1999 Academic Press.

TI Comparison of **SH3** and SH2 Domain Dynamics when Expressed Alone or in an SH(3+2) Construct: The Role of Protein Dynamics in Functional Regulation

L11 ANSWER 12 OF 34 CA COPYRIGHT 2001 ACS

AB Hematopoietic cell kinase (**Hck**) is a member of the Src-family of protein tyrosine kinases. We have found that upon enzymic activation of **Hck** by the heavy metal mercuric chloride, there was a rapid increase in the levels of tyrosine phosphorylation of several proteins including the proto-oncogene p120Cbl. Fibroblasts that are transformed with an activated allele of **Hck** exhibit constitutive Cbl phosphorylation. Upon Fc.gamma. receptor activation, a more physiol. relevant extracellular signal, Cbl is tyrosine phosphorylated and the Src-family selective inhibitor, PP1, can prevent this phosphorylation on Cbl. **Hck** phosphorylates Cbl in vitro and the interaction between Cbl and **Hck** is direct, requiring **Hck**'s unique, **SH3** and SH2 domains for optimal binding. Using a novel estrogen-regulated chimera of **Hck**, we have shown a hormone-dependent assocn. between **Hck** and Cbl in murine fibroblasts. This work suggests that Cbl serves as a key mediator of **Hck** induced signalling in hematopoietic cells. (c) 1999 Academic Press.

TI The Proto-oncogene p120Cbl Is a Downstream Substrate of the **Hck** Protein-Tyrosine Kinase

L11 ANSWER 13 OF 34 CA COPYRIGHT 2001 ACS

AB We have investigated the interaction between Cbl and the Src-related tyrosine kinase Fyn. Fyn was obsd. to be constitutively assocd. with Cbl in lysates of several different cell types including the interleukin-3-dependent murine myeloid cell line 32Dc13, and the prolactin-dependent rat thymoma cell line Nb2. Binding studies indicated that Cbl could bind to glutathione S-transferase (GST) fusion proteins encoding the unique, Src homol. domain 3 (**SH3**), and SH2 domains of Fyn, **Hck**, or Lyn. Fusion proteins encoding either the **SH3** or SH2 domains of Fyn bound to Cbl as effectively as the fusion protein encoding the unique, **SH3**, and SH2 domains of Fyn. The Fyn SH2 domain bound to both tyrosine-phosphorylated and nonphosphorylated Cbl, implying that this interaction might be phosphotyrosine-independent. Binding of the Fyn SH2 domain to Cbl was not disrupted by the addn. of phosphotyrosine, phosphoserine, or phosphothreonine. A GST fusion protein encoding the proline-rich region of Cbl bound to Fyn present in a total cell lysate. Far Western blot anal. also indicated that the **SH3** domain of Fyn bound preferentially to the proline-rich region of Cbl. The addn. of [.gamma.-32P]ATP to either anti-Cbl immunoppts. or anti-Fyn immunoppts. resulted in the phosphorylation of both Cbl and Fyn as demonstrated by immunopptn. of the phosphorylated proteins with specific antisera. Fyn directly phosphorylated a GST fusion protein contg. the C-terminal region of Cbl (GST-CBL-LZIP). In contrast, immunopptd. JAK2 was not able to phosphorylate this same region of Cbl. The GST-CBL-LZIP fusion protein contains a binding site for the SH2 domain of the p85 subunit of phosphatidylinositol 3-kinase, which mapped to Tyr731, which is present in the sequence YEAM. Mutation of Tyr731 in GST-CBL-LZIP eliminated binding of the p85 subunit of phosphatidylinositol 3-kinase and substantially reduced the phosphorylation of this fusion protein by Fyn, despite the

presence of four other tyrosine residues in this fusion protein. These data are consistent with the hypothesis that Cbl represents a substrate for Src-like kinases that are activated in response to the engagement of cell surface receptors, and that Src-like kinases are responsible for the phosphorylation of a tyrosine residue in Cbl that may regulate activation of phosphatidylinositol 3-kinase.

TI Fyn associates with Cbl and phosphorylates tyrosine 731 in Cbl, a binding site for phosphatidylinositol 3-kinase

L11 ANSWER 14 OF 34 CA COPYRIGHT 2001 ACS

AB Fc receptors modulate inflammatory processes, including phagocytosis, serotonin and histamine release, superoxide prodn., and secretion of cytokines. Aggregation of Fc.gamma.RIIa, the low-affinity receptor for monomeric IgG, activates nonreceptor protein tyrosine kinases such as Lyn, **Hck**, and Syk, potentially driving the phosphorylation of the downstream adaptor proteins, including Cbl and/or Nck. Previous work from our lab. using interferon-.gamma.-differentiated U937 (U937IF) myeloid cells investigated mechanisms which regulate Fc.gamma. receptor-induced assembly of adaptor complexes. Herein, we report that Fc.gamma.RII receptor signaling in U937IF and HEL cells involves Cbl and Nck, suggesting that Cbl-Nck interactions may link Fc.gamma.RII to downstream activation of Pak kinase. Fc.gamma.RII crosslinking induced the phosphorylation of Cbl and Nck on tyrosine. The .alpha.Cbl immunopptns. revealed constitutive binding of Nck and Grb2 to Cbl and Fc.gamma.RII-inducible binding of CrkL to Cbl. The interactions of Cbl with Nck and CrkL were phosphorylation dependent since dephosphorylation of cellular proteins with potato acid phosphatase abrogated binding. GST-Nck fusion protein pulldown expts. show that Cbl and Pak1 bind to the second **SH3** domain of Nck. A specific Src inhibitor, PP1, was shown to completely abrogate the Fc.gamma.R-induced superoxide response, correlating with a decrease in Cbl and Nck tyrosine phosphorylation. Our results provide the first evidence that Src is required for Fc.gamma.R activation of the respiratory burst in myeloid cells and suggest that Cbl-Nck, Cbl-Pak1, and Nck-Pak1 interactions may regulate this response. (c) 1998 Academic Press.

TI Characterization of Cbl-Nck and Nck-Pak1 interactions in myeloid Fc.gamma.RII signaling

L11 ANSWER 15 OF 34 CA COPYRIGHT 2001 ACS

AB Intramol. interactions between the Src homol. domains (SH2 and **SH3**) and the catalytic domains of Src family kinases result in repression of catalytic activity. The crystal structure of the Src family kinase **Hck**, with its regulatory domains intact, has been solved. It predicts that a conserved residue, Trp260, at the end of the linker between the SH2 and the catalytic domains plays an important role in regulation by the **SH3** and SH2 domains. We have mutated this residue and compared the activities of C-terminally phosphorylated wild type **Hck** and W260A **Hck**. The W260A mutant has a higher specific activity than wild type **Hck**. The W260A mutant requires autophosphorylation at Tyr416 for full activity, but it is not activated by ligand binding to the **SH3** or SH2 domains. This mutation also changes the accessibility of the SH2 and **SH3** domains to their cognate peptide ligands. Our results indicate that Trp260 plays a crit. role in the coupling of the regulatory domains to the catalytic domain, as well as in positioning the ligand binding surfaces.

TI Intramolecular regulatory interactions in the Src family kinase **Hck** probed by mutagenesis of a conserved tryptophan residue

L11 ANSWER 16 OF 34 CA COPYRIGHT 2001 ACS

AB The Nef gene of the human and simian immunodeficiency viruses HIV and SIV has been implicated in pathogenicity; however, the mechanism by which Nef induces disease is still unknown. An impact on signal transduction in

cells has been suggested by the interaction of Nef from an HIV-1 strain and tyrosine kinases like **HCK** and LCK as well as serine/threonine kinases. We have confirmed the binding of **HCK** to HIV-1 subtype B Nef and demonstrated an equally strong interaction with a subtype E Nef protein but weaker binding to Nef of HIV-2 subtype A (HIV-2D194). No binding, however, was obsd. to HIV-2 subtype B Nef (HIV-2D205). Instead, this protein bound to a novel cellular protein, Nefin 1, with characteristics of an adaptor protein and strong expression in all human hematopoietic tissues. Nefin 1 binds through an amino-terminal domain, which is related to **SH3** domains. For interaction of Nef with Nefin 1, the PxxP motif and the three-dimensional conformation of the mol. appear necessary. In conclusion, this study demonstrates that Nef proteins of divergent strains of HIV-1 and HIV-2 may use different elements of signal transduction pathways for the induction of pathogenicity in vivo. (c) 1998 Academic Press.

TI Nef proteins of distinct HIV-1 or -2 isolates differ in their binding properties for **HCK**: isolation of a novel Nef binding factor with characteristics of an adaptor protein

L11 ANSWER 17 OF 34 CA COPYRIGHT 2001 ACS

AB The interaction between the Human Immunodeficiency Virus Nef protein (HIV-1 Nef) and the Src Homol. Region 3 (**SH3**) domain of **Hck** was studied using scintillation proximity assay (SPA). SPA is a quick and sensitive method that does not require a sepn. step, thus allowing assays to be performed in a homogeneous environment. In contrast to most conventional techniques, SPA may also be used to detect low affinity protein-protein interactions. In this study, the assay was configured using biotinylated **Hck SH3** domain expressed both as a GST fusion protein and synthesized chem. in its' native form. Biotinylated **Hck** protein was immobilized to streptavidin-coated fluoromicrosphere SPA beads and the binding of [3H]Nef was detected by scintillation counting. Anal. of binding yielded an av. equil. dissocn. const. (KD) of 183 .+-. 30 nM for the interaction in line with reported values by other methods. The data presented demonstrates that using SPA, protein-protein interactions of relatively low affinity can be detected with a high degree of sensitivity and screening studies of inhibitors of these assocns. could be facilitated by the high sample throughput achievable with SPA.

TI Screening assay for the detection of the protein-protein interaction between HIV-1 Nef protein and the **SH3** domain of **Hck**

L11 ANSWER 18 OF 34 CA COPYRIGHT 2001 ACS

AB **SH3** domains are protein binding domains that occur widely among signal transduction proteins. Here, we present the NMR-detd. soln. structure of the **SH3** domain from the cytoplasmic protein tyrosine kinase, **Hck**. **Hck** is involved in a no. of cell signal transduction pathways, frequently in pathways assocd. with immune response. **SH3** domains bind proteins via a left-handed polyproline type II helix on the target protein. We have assessed the structural impact of binding to a ligand through addn. of a peptide corresponding to a proline-rich region of a **Hck** target, the GTPase activating protein of the Ras pathway. Ligand binding effects small structural changes and stabilizes the **SH3** domain structure. Also, we have compared the soln. structure of the **Hck SH3** domain to the crystal structure of **Hck**, in which the **SH3** domain exhibits an intramol. binding to an interdomain linker region. These structures are interpreted as the apo- and holo-forms of the **Hck SH3** domain.

TI Solution structure of the human **Hck SH3** domain and identification of its ligand binding site

L11 ANSWER 19 OF 34 CA COPYRIGHT 2001 ACS

AB The Src protein tyrosine kinase plays a crit. role in a variety of signal transduction pathways. Strict regulation of its activity is necessary for proper signaling. We present here the crystal structure of chicken Src which is phosphorylated at Tyr527 and represents its least active form. Our structure, similar to the recently reported human **Hck** and Src structures, contains the **SH3**, SH2 and the kinase domains and the C-terminal regulatory tail but not the N-terminal unique domain. The **SH3** domain uses its hydrophobic surface to coordinate the SH2-kinase linker such that residues Gln251 and Leu255 specifically interact with side chains in the .beta.2-.beta.3 and the .alpha.C-.beta.4 loops of the N-terminal lobe opposite of the kinase active site. This position of the **SH3** domain and the coordination of the SH2-kinase linker also optimally places the SH2 domain such that the phosphorylated Tyr527 in the C-terminal tail interacts with the SH2 binding pocket. Analogous to Cdk2 kinase, the position of the Src .alpha.C-helix in the N-terminal lobe is swung out disrupting the position of the active site residues. Superposition of other protein kinases including human **Hck** and Src onto chicken Src indicate that the .alpha.C-helix position is affected by the relative position of the N-terminal lobe with respect to the C-terminal lobe of the kinase and that the presence of the **SH3**/SH2-kinase linker/N-terminal lobe interactions restricts the kinase lobes and .alpha.C-helix access to the active conformation. These superpositions also suggest that the highly conserved .alpha.C-.beta.4 loop restricts the conformational freedom of the N-terminal lobe by anchoring it to the C-terminal lobe. Finally, based on sequence alignments and conservation of hydrophobic residues in the Src SH2-kinase linker as well as in the .alpha.C-.beta.4 and .beta.2-.beta.3 loops, we propose that the Src-related kinases, Abl, Btk and Csk, share the same quaternary structure.

TI The 2.35 .ANG. crystal structure of the inactivated form of chicken Src: a dynamic molecule with multiple regulatory interactions

L11 ANSWER 20 OF 34 CA COPYRIGHT 2001 ACS

AB The crystal structures of the regulated Src and **Hck** tyrosine kinases show intramol. interactions between the phosphorylated tail and the SH2 domain as well as between the **SH3** domain, the SH2-catalytic domain linker (SH2-CD linker) and the catalytic domain. The relative contribution of these interactions to regulation of activity is poorly understood. Mutational anal. of Src and Lck revealed that interaction of the SH2-CD linker with the **SH3** domain is crucial for regulation. Moreover, three sites of interaction of the linker with the catalytic domain, one at the beginning (Trp 260) and two at the back of the small lobe, opposite the catalytic cleft (.beta.2/.beta.3 loop; .alpha.C/.beta.4 loop), impinge on Src activity. Other activating mutations map to the front of the catalytic domain in the loop preceding the .alpha.C-helix (.beta.3/.alpha.C loop). SH2-CD linker mutants are deregulated in mammalian cells but transform fibroblasts weakly, suggesting that the linker may bind cellular components. The authors' results, on the basis of the crystal structure of Src, favor a model in which the correctly positioned SH2-CD linker exerts an inhibitory function on catalysis of Src family members by facilitating displacement of the .alpha.C-helix. This study may provide a template for the generation of deregulated versions of other protein kinases.

TI The role of the linker between the SH2 domain and catalytic domain in the regulation and function of Src

L11 ANSWER 21 OF 34 CA COPYRIGHT 2001 ACS

AB A review with 45 refs. The crystal structures of 3 Src-family protein tyrosine kinases have been detd. recently. The structure of the catalytic

domain of Lck has been detd. in the active autophosphorylated state. The structures of larger constructs of c-Src and **Hck**, contg. the **SH3**, SH2 and catalytic domains, as well as a C-terminal regulatory tail, have been detd. in the down-regulated state, phosphorylated in the C-terminal tail. A comparison of these structures leads to an unanticipated mechanism for the regulation of catalytic activity by cooperative interactions between the SH2, **SH3**, and catalytic domains.

TI Structures of Src-family tyrosine kinases

L11 ANSWER 22 OF 34 CA COPYRIGHT 2001 ACS

AB A review with 114 refs., of the structural basis for the binding of phosphotyrosine peptides to SH2 domains and proline-rich peptides to **SH3** domains. The SH2 domain contains a conserved basic pocket which binds the phosphotyrosine moiety and a 2nd less conserved pocket or groove which is responsible for conferring specificity by binding hydrophobic residues C-terminal from the phosphotyrosine. The **SH3** domains contain a rather flat hydrophobic surface that recognizes proline-rich peptides, adopting a left-handed proline type-II helix. The recently detd. structures of the protein tyrosine kinases, c-Src and **Hck**, show how SH2 and **SH3** domains can regulate the function of a protein tyrosine kinase.

TI Structural analysis of the SH2 and **SH3** domains: modules that regulate protein interactions

L11 ANSWER 23 OF 34 CA COPYRIGHT 2001 ACS

AB Tyrosine kinases of the Src family are regulated via their Src homol. 2 (SH2) and **SH3** domains. The Nef protein of human immunodeficiency virus-1 (HIV-1) has previously been shown to bind with high affinity and specificity in vitro to the **SH3** domain of **Hck**, a Src family member expressed primarily in myeloid cells. However, the effect of Nef on **Hck** activity in living cells. However, the effect of Nef on **Hck** activity in living cells is unknown. Here we show that Rat-2 fibroblasts coexpressing **Hck** and Nef rapidly developed transformed foci, whereas control cells expressing either protein alone did not. Nef formed a stable complex with **Hck** and stimulated its tyrosine kinase activity in vivo. Mutagenesis of the Nef proline-rich motif essential for **SH3** binding completely blocked complex formation, kinase activation, and transformation, indicating that the Nef **SH3**-bining function is required for its effects on **Hck**. These results provide direct evidence that **SH3** engagement is sufficient to activate a Src family kinase in vivo and suggest that **Hck** may be activated by Nef in HIV-infected macrophages.

TI **SH3**-mediated **Hck** tyrosine kinase activation and fibroblast transformation by the Nef protein of HIV-1

L11 ANSWER 24 OF 34 CA COPYRIGHT 2001 ACS

AB We have previously shown that stimulation of 32D cl3 cells with interleukin (IL)-3 results in the activation of three src-like tyrosine kinases, fyn, **hck**, and lyn. The .beta. subunit of the IL-3 receptor co-immunopptd. with **hck** in lysates of both unstimulated and IL-3-stimulated cells; however, the .beta. subunit did not ppt. with either fyn or lyn. The assocn. of these three kinases with the .beta. subunit of the IL-3 receptor was further investigated using bacterial fusion proteins encoding the unique, **SH3**, and SH2 domains of these three kinases. Fusion proteins of both **hck** and fyn bound to a 150-kDa tyrosine-phosphorylated protein present in lysates of IL-3-stimulated cells. This protein was identified as the .beta. subunit of the IL-3 receptor by immunoblotting with an anti-.beta. antibody. Glutathione S-transferase (GST) fusion proteins contg. the SH2 domain of **hck** bound to the .beta. subunit although the amt. of .beta. subunit that bound to the SH2 domain alone was only 30% of that which bound to the fusion protein contg. the unique, **SH3**, and SH2

domains. This indicates that the SH2 domain is one of the motifs involved in binding **hck** to the .beta. subunit. A GST fusion protein encoding a 236-amino acid region of the cytoplasmic tail of the .beta. subunit, which contained for tyrosine residues, bound to **hck** and fyn. Binding to both proteins was dramatically increased when the GST-.beta. fusion protein was tyrosine-phosphorylated. Far Western blot anal. was used to demonstrate the binding of the unique, **SH3**, and SH2 domains of **hck** to this 236-amino acid region of the .beta. subunit; tyrosine phosphorylation of this protein increased the binding of both the unique region and the SH2 domain probes. These data indicate that binding of **hck** to the .beta. subunit is mediated by both phosphotyrosine-dependent and -independent mechanisms.

TI Binding of src-like kinases to the .beta.-subunit of the interleukin-3 receptor

L11 ANSWER 25 OF 34 CA COPYRIGHT 2001 ACS

AB The tendency of HIV-1 Nef to form aggregates in soln., particularly at pH values below 8, together with its large fraction of highly mobile residues seriously complicated detn. of its three-dimensional structure, both for heteronuclear soln. NMR (Grzesiek et al., 1996a, Nat Struct Biol 3:340-345) and for x-ray crystallog. (Lee et al., 1996, Cell 85:931-942). Methods used to det. the Nef structure by NMR at pH 8 and 0.6 mM concn. are presented, together with a detailed description of Nef's secondary and tertiary structure. The described techniques have general applicability for the NMR structure detn. of proteins that are aggregating and/or have limited stability at low pH values. Extensive chem. shift assignments are reported for backbone and side chain 1H, 13C, and 15N resonances of the HIV-1 Nef deletion mutants NEF.DELTA.2-39, NEF.DELTA.2-39,.DELTA.159-173, and of NEF.DELTA.2-39,.DELTA.159-173 in complex with the **SH3** domain of the **Hck** tyrosine protein kinase. Besides a type II polyproline helix, Nef's structure consists of three .alpha.-helixes, a 310 helix, and a five-stranded anti-parallel .beta.-sheet. The anal. of 15N relaxation parameters of the backbone amide sites reveals that all the secondary structure elements are non-mobile on the picosecond to nanosecond and on the millisecond time scale. A large no. of slowly exchanging amide protons provides evidence for the stability of the Nef core even on the time scale of hours. Significant internal motions on the ps to ns time scale are detected for residues 60 to 71 and for residues 149 to 180, which form solvent-exposed loops. The residues of the HIV-1 protease cleavage site (W57/L58) do not exhibit large amplitude motions on the sub-nanosecond time scale, and their side chains insert themselves into a hydrophobic crevice formed between the C-terminus of helix 1 and the N-terminus of helix 2. A refined structure has been detd. based on addnl. constraints for side-chain and backbone dihedral angles derived from a large no. of three-bond J-couplings and ROE data.

TI Refined solution structure and backbone dynamics of HIV-1 Nef

L11 ANSWER 26 OF 34 CA COPYRIGHT 2001 ACS

AB The crystal structure of the hematopoietic cell kinase **Hck** has been detd. at 2.6/2.9 .ANG. resoln. Inhibition of enzymic activity is a consequence of intramol. interactions of the enzyme's Src-homol. domains SH2 and **SH3**, with concomitant displacement of elements of the catalytic domain. The conformation of the active site has similarities with that of inactive cyclin-dependent protein kinases.

TI Crystal structure of the Src family tyrosine kinase **Hck**

L11 ANSWER 27 OF 34 CA COPYRIGHT 2001 ACS

AB The protein **Hck** is a member of the Src family of non-receptor

tyrosine kinases which is preferentially expressed in hematopoietic cells of the myeloid and B-lymphoid lineages. Src kinases are inhibited by tyrosine-phosphorylation at a carboxy-terminal site. The SH2 domains of these enzymes play an essential role in this regulation by binding to the tyrosine-phosphorylated tail. The crystal structure of the downregulated form of **Hck** has been detd. and reveals that the SH2 domain regulates enzymic activity indirectly; intramol. interactions between the **SH3** and catalytic domains appear to stabilize an inactive form of the kinase. Here, we compare the roles of the SH2 and **SH3** domains in modulating the activity of **Hck** in an investigation of the C-terminally phosphorylated form of the enzyme. We show that addn. of the HIV-1 Nef protein, which is a high-affinity ligand for the **Hck** **SH3** domain, to either the downregulated or activated form of **Hck** causes a large increase in **Hck** catalytic activity. The intact **SH3**-binding motif in Nef is crucial for **Hck** activation. Our results indicate that binding of the **Hck** **SH3** domain by Nef causes a more marked activation of the enzyme than does binding of the SH2 domain, suggesting a new mechanism for regulation of the activity of tyrosine kinases.

TI Activation of the Src-family tyrosine kinase **Hck** by **SH3** domain displacement

L11 ANSWER 28 OF 34 CA COPYRIGHT 2001 ACS

AB We have demonstrated that a 120-kDa protein, identified as Cbl, becomes rapidly phosphorylated on tyrosine residues following stimulation of factor-dependent cells with interleukin-3 (IL-3). Little or no phosphorylation of Cbl was obsd. in the absence of IL-3 stimulation and phosphorylation is maximal by 20-30 min after IL-3 stimulation. Assocn. of Cbl with Grb2 was noted in unstimulated cells, and the amt. of Cbl assocd. with Grb2 increased following IL-3 stimulation. The p85 subunit of phosphatidylinositol 3-kinase was constitutively assocd. with Cbl. Approx. 10% of the PI kinase activity present in anti-phosphotyrosine immunoppts. was present in anti-Cbl immunoppts. of IL-3-stimulated cells. The constitutive assocn. of Cbl with Fyn was also obsd. Cbl was obsd. to bind to bacterial fusion proteins encoding the unique, **SH3**, and SH2 domains of Fyn, **Hck**, and Lyn. The SH2 domain of Fyn alone was able to bind Cbl to nearly the same extent as did the fusion protein encoding the unique, **SH3**, and SH2 domains. This was not the case for the SH2 domain of **Hck**, however, as binding of the **Hck** fusion protein to Cbl appeared to require multiple domains. The binding of the fusion proteins to Cbl occurred regardless of whether Cbl was tyrosine-phosphorylated or not, and the binding could not be disrupted by the addn. of 30 mM free phosphotyrosine. These data suggest the unexpected conclusion that the Fyn SH2 domain may bind to Cbl in a phosphotyrosine-independent manner.

TI Phosphorylation of Cbl following stimulation with interleukin-3 and its association with Grb2, Fyn, and phosphatidylinositol 3-kinase

L11 ANSWER 29 OF 34 CA COPYRIGHT 2001 ACS

AB Using heteronuclear NMR spectroscopy, the authors demonstrate that a 13-residue peptide (MSQIKRLLSEKKT) from the cytoplasmic tail of CD4 binds to Nef protein. This part of CD4 is crit. for downregulation of CD4 by HIV-1 Nef (Aiken et al., 1994). The authors show that a control peptide without the central dileucine does not bind to Nef. The dependence of Nef 1H and 15N amide chem. shifts on peptide concn. indicates that the binding is in the fast chem. exchange limit, with a dissocn. const. Kd of .apprx.1 mM. The peptide binding site has been mapped onto the previously detd. soln. structure of HIV-1 Nef (Grzesiek et al., 1996) on the basis of peptide-induced chem. shift changes. It comprises amino acids W57, L58, E59, G95, G96, L97, R106, and L110. When Nef is complexed to the **SH3** domain of **Hck** tyrosine protein kinase, the peptide

binds to the same site on Nef but with slightly higher affinity (Kd .apprx. 0.5 mM). Thus, the binding of CD4 and **Hck SH3** to Nef are two compatible and slightly cooperative events.

TI The CD4 Determinant for Downregulation by HIV-1 Nef Directly Binds to Nef.

Mapping of the Nef Binding Surface by NMR

L11 ANSWER 30 OF 34 CA COPYRIGHT 2001 ACS

AB The soln. structure of HIV-1 Nef has been solved by multidimensional heteronuclear NMR spectroscopy. The construct employed to circumvent problems assocd. with aggregation was a double-deletion mutant (.DELTA.2-39,.DELTA.159-173) in which conformationally disordered regions of the protein at the N terminus and in a long solvent-exposed flexible loop were removed, without affecting the properties or structural integrity of the remainder of the protein. Despite the absence of any sequence similarity, the overall fold of Nef is reminiscent of that of the family of winged helix-turn-helix DNA binding proteins. The binding surface of Nef for the **SH3** domain of **Hck** tyrosine protein kinase has been mapped and reveals a non-contiguous (in terms of amino-acid sequence) interaction surface. This unique feature may suggest possible avenues for drug design aimed at inhibiting the interaction between Nef and **SH3** domains.

TI The solution structure of HIV-1 Nef reveals an unexpected fold and permits delineation of the binding surface for the **SH3** domain of **Hck** tyrosine protein kinase

L11 ANSWER 31 OF 34 CA COPYRIGHT 2001 ACS

AB Middle-T antigen of mouse polyomavirus, an oncogenic DNA virus, assocs. with and activates the cellular tyrosine kinases c-Src, c-Yes, and Fyn. This interaction is essential for polyomavirus-mediated transformation of cells in culture and tumor formation in animals. To det. the domain of c-Src directing assocn. with middle-T, mutant c-Src proteins lacking either the amino-terminal unique domain and the myristylation signal, the SH2 domain, the **SH3** domain, or all three of these domains were coexpressed with middle-T in NIH 3T3 cells. All mutants were found to assoc. with middle-T, demonstrating that the kinase domain of c-Src, including the carboxy-terminal regulatory tail, is sufficient for assocn. with middle-T. Moreover, we found that **Hck**, another membrane of the Src kinase family, does not bind middle-T, while chimeric kinases consisting of the amino-terminal domains of c-Src fused to the kinase domain of **Hck** or the amino-terminal domains of **Hck** fused to the kinase domain of c-Src assocd. with middle-T. **Hck** mutated at its carboxy-terminal regulatory residue, tyrosine 501, was also found to assoc. with middle-T. These results suggest that in **Hck**, the postulated intramol. interaction between the carboxy-terminal regulatory tyrosine and the SH2 domain prevents assocn. with middle-T. This intramol. interaction apparently also limits the ability of c-Src to assoc. with middle-T, since removal of the SH2 or **SH3** domain increases the efficiency with which middle-T binds c-Src.

TI Polyomavirus middle-T antigen associates with the kinase domain of Src-related tyrosine kinases

L11 ANSWER 32 OF 34 CA COPYRIGHT 2001 ACS

AB Products and processes are disclosed for regulating signal transduction pathways in cells. One aspect of the invention relates to a peptide having a YXXLXXXXXXXYXX.psi. motif (X = amino acid; .psi.= Leu, Ile) that is useful in regulating the activity of tyrosine kinases, lipid kinases and adaptor mols. A sep. aspect of the present invention relates to a product and process for inhibiting signal transduction pathways in cells involving a peptide capable of binding to an **SH3** domain of a tyrosine kinase, thereby blocking the binding and activation of an

effector by the tyrosine kinase. Both the above compd. and peptide compn.
can be useful in the treatment of medical disorders such as allergic responses, autoimmune diseases, inflammatory responses, cancer, immunodeficiency diseases, immuno-proliferative diseases and diseases caused by viruses, such as Epstein-Barr virus and bovine leukemia virus.
TI Product and process for regulating signal transduction pathways

L11 ANSWER 33 OF 34 CA COPYRIGHT 2001 ACS
AB A review with 65 refs. The c-src gene family has nine known members (blk,
c-fgr, fyn, **hck**, lck, lyn, c-src, c-yes and yrk), each encoding a cytoplasmic protein-tyrosine kinase (PTK) believed to be involved in signal transduction. The c-src PTKs contain three domains (SH1, SH2 and **SH3**) that are found in many other signalling proteins. The SH1 domain has PTK activity, while the SH2 and **SH3** domains are involved in mediating protein-protein interactions by binding to phosphotyrosine-contg. and proline-rich motifs, resp. The expression patterns of the c-src PTKs suggest that they function in a broad range of biol. situations, in many cases regulating the behavior of terminally-differentiated, post-mitotic cell types. Targeted disruption of members of the c-src family in transgenic mice has confirmed important roles for p56lck and p59fyn(T) in T-lymphocyte maturation and activation, but has also revealed unexpected roles for p60c-src in bone maintenance and for p59fyn(B) in learning and memory. There is increasingly detailed information about the biochem. nature of the signalling pathways in which the c-src PTKs operate and about the other signalling proteins with which they interact. The c-src PTKs can assoc. with activated receptor PTKs, including the receptors for platelet-derived growth factor and epidermal growth factor, by means of SH2-phosphotyrosine binding. The c-src PTKs also assoc. with transmembrane proteins that lack PTK activity, frequently
by means of interactions involving their unique amino-terminal sequences.
TI Signalling by the p60c-src family of protein-tyrosine kinases

L11 ANSWER 34 OF 34 CA COPYRIGHT 2001 ACS
AB The Ras GTPase-activating protein (GAP) is a target for protein tyrosine kinases of both the receptor and cytoplasmic classes and may serve to integrate tyrosine kinase and Ras signaling pathways. Here, the authors provide evidence that GAP is an **SH3** domain-binding protein and substrate for the Src-related protein tyrosine kinase **Hck**, which has been implicated in the regulation of myeloid cell growth, differentiation, and function. Wild-type (WT) or kinase-inactive (K269E) mutant **Hck** proteins were coexpressed with bovine GAP using the baculovirus/Sf9 cell system. GAP was readily phosphorylated on tyrosine by WT but not K269E **Hck**. GAP was present in WT **Hck** immunoppts. from the coinfected cells, indicative of **Hck** .cntdot.GAP complex formation. Unexpectedly, GAP also assocd. with the kinase-inactive mutant of **HcK**, suggesting that tyrosine autophosphorylation of **HcK** is not required for complex formation. The WT and K269E forms of **Hck** also assocd. with GAP mutants lacking either the C-terminal catalytic domain (.DELTA.CAT) or the
Src homol. region (.DELTA.SH), indicating that these GAP domains are dispensable for complex formation. Recombinant GST fusion proteins contg.
the **Hck**, Src, Fyn, or Lck **SH3** domains assocd. with full-length GAP, .DELTA.CAT, and .DELTA.SH, all of which share an N-terminal proline-rich region resembling an **SH3**-binding motif (PPLPPPPPQLP). Deletion of the highly conserved YXY sequence from the **Hck SH3** domain abolished binding. GAP-**SH3** interaction was also inhibited by proline-rich peptide GFPPLPPPPPQLPTLG, which corresponds to N-terminal amino acids 129-144 of bovine GAP. An N-terminal deletion mutant of GAP lacking this proline-rich region did not

bind to the **Hck SH3** domain. These data implicate the **Hck SH3** domain in GAP interaction, and suggest a general function for the **SH3** domains of Src family kinases in recognition of GAP via its proline-rich N-terminal domain.

TI The Ras GTPase-activating protein (GAP) is an **SH3** domain-binding protein and substrate for the Src-related tyrosine kinase, **Hck**

```
=> d his

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     FILE 'CA' ENTERED AT 13:55:23 ON 09 APR 2001
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L2           54 S L1 AND SH3
L3            7 S L2 AND LIBRARY
L4           47 S L2 NOT L3
L5            3 S L4 AND RT LOOP
L6           44 S L4 NOT L5
L7            0 S L6 AND (ASP-ALA-ISO-HIS-HIS-GLU)
L8            0 S L6 AND PLASMIDS
L9            0 S L6 AND PHAGEMID
L10          10 S L6 AND VIRAL
L11          34 S L6 NOT L10

=> d l11 1-34 py au so

L11  ANSWER 1 OF 34  CA  COPYRIGHT 2001 ACS
PY   2000
AU   Engen, John R.; Smith, David L.
SO   Methods Mol. Biol. (Totowa, N. J.) (2000), 146(Mass Spectrometry of
     Proteins and Peptides), 95-112
     CODEN: MMBIED; ISSN: 1064-3745

L11  ANSWER 2 OF 34  CA  COPYRIGHT 2001 ACS
PY   2000
AU   Scott, Margaret Porter; Miller, W. Todd
SO   Biochemistry (2000), 39(47), 14531-14537
     CODEN: BICHAW; ISSN: 0006-2960

L11  ANSWER 3 OF 34  CA  COPYRIGHT 2001 ACS
PY   2000
AU   Nguyen, Jack T.; Porter, Margaret; Amoui, Mehran; Miller, W. Todd;
     Zuckermann, Ronald N.; Lim, Wendell A.
SO   Chem. Biol. (2000), 7(7), 463-473
     CODEN: CBOLE2; ISSN: 1074-5521

L11  ANSWER 4 OF 34  CA  COPYRIGHT 2001 ACS
PY   2000
AU   Lionberger, Jack M.; Wilson, Matthew B.; Smithgall, Thomas E.
SO   J. Biol. Chem. (2000), 275(24), 18581-18585
     CODEN: JBCHA3; ISSN: 0021-9258

L11  ANSWER 5 OF 34  CA  COPYRIGHT 2001 ACS
PY   2000
AU   Scholz, Glen; Cartledge, Kellie; Dunn, Ashley R.
SO   J. Biol. Chem. (2000), 275(19), 14615-14623
     CODEN: JBCHA3; ISSN: 0021-9258

L11  ANSWER 6 OF 34  CA  COPYRIGHT 2001 ACS
PY   2000
AU   Gerhardstein, Brian L.; Gao, Tianyan; Bunemann, Moritz; Puri, Tipu S.;
     Adair, Adam; Ma, Hong; Hosey, M. Marlene
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SO J. Biol. Chem. (2000), 275(12), 8556-8563
CODEN: JBCHA3; ISSN: 0021-9258

L11 ANSWER 7 OF 34 CA COPYRIGHT 2001 ACS
PY 2000
AU Horita, David A.; Zhang, Weixing; Smithgall, Thomas E.; Gmeiner, William H.; Byrd, R. Andrew
SO Protein Sci. (2000), 9(1), 95-103
CODEN: PRCIEI; ISSN: 0961-8368

L11 ANSWER 8 OF 34 CA COPYRIGHT 2001 ACS
PY 2000
AU Porter, Margaret; Schindler, Thomas; Kuriyan, John; Miller, W. Todd
SO J. Biol. Chem. (2000), 275(4), 2721-2726
CODEN: JBCHA3; ISSN: 0021-9258

L11 ANSWER 9 OF 34 CA COPYRIGHT 2001 ACS
PY 1999
AU Briggs, Scott D.; Smithgall, Thomas E.
SO J. Biol. Chem. (1999), 274(37), 26579-26583
CODEN: JBCHA3; ISSN: 0021-9258

L11 ANSWER 10 OF 34 CA COPYRIGHT 2001 ACS
PY 1999
AU Schindler, Thomas; Sicheri, Frank; Pico, Alexander; Gazit, Aviv; Levitzki,
Alexander; Kuriyan, John
SO Mol. Cell (1999), 3(5), 639-648
CODEN: MOCEFL; ISSN: 1097-2765

L11 ANSWER 11 OF 34 CA COPYRIGHT 2001 ACS
PY 1999
AU Engen, John R.; Smithgall, Thomas E.; Gmeiner, William H.; Smith, David L.
SO J. Mol. Biol. (1999), 287(3), 645-656
CODEN: JMOBAK; ISSN: 0022-2836

L11 ANSWER 12 OF 34 CA COPYRIGHT 2001 ACS
PY 1999
AU Howlett, Christopher J.; Bisson, Sabine A.; Resek, Mary E.; Tigley, Allan W.; Robbins, Stephen M.
SO Biochem. Biophys. Res. Commun. (1999), 257(1), 129-138
CODEN: BBRCA9; ISSN: 0006-291X

L11 ANSWER 13 OF 34 CA COPYRIGHT 2001 ACS
PY 1999
AU Hunter, Seija; Burton, Elizabeth A.; Wu, Steven C.; Anderson, Steven M.
SO J. Biol. Chem. (1999), 274(4), 2097-2106
CODEN: JBCHA3; ISSN: 0021-9258

L11 ANSWER 14 OF 34 CA COPYRIGHT 2001 ACS
PY 1998
AU Izadi, Kayvon D.; Erdreich-Epstein, Anat; Liu, Yenbou; Durden, Donald L.
SO Exp. Cell Res. (1998), 245(2), 330-342
CODEN: ECREAL; ISSN: 0014-4827

L11 ANSWER 15 OF 34 CA COPYRIGHT 2001 ACS
PY 1998
AU LaFevre-Bernt, Michelle; Sicheri, Frank; Pico, Alexander; Porter, Margaret; Kuriyan, John; Miller, W. Todd
SO J. Biol. Chem. (1998), 273(48), 32129-32134
CODEN: JBCHA3; ISSN: 0021-9258

L11 ANSWER 16 OF 34 CA COPYRIGHT 2001 ACS
PY 1998

AU Karn, Thomas; Hock, Bjorn; Holtrich, Uwe; Adamski, Michalina; Strebhardt, Klaus; Rubsamen-Waigmann, Helga
SO Virology (1998), 246(1), 45-52
CODEN: VIRLAX; ISSN: 0042-6822

L11 ANSWER 17 OF 34 CA COPYRIGHT 2001 ACS
PY 1998
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SO J. Biomol. Screening (1998), 3(1), 37-41
CODEN: JBISF3; ISSN: 1087-0571

L11 ANSWER 18 OF 34 CA COPYRIGHT 2001 ACS
PY 1998
AU Horita, David A.; Baldisseri, Donna M.; Zhang, Weixing; Altieri, Amanda S.; Smithgall, Thomas E.; Gmeiner, William H.; Byrd, R. Andrew
SO J. Mol. Biol. (1998), 278(1), 253-265
CODEN: JMOBAK; ISSN: 0022-2836

L11 ANSWER 19 OF 34 CA COPYRIGHT 2001 ACS
PY 1997
AU Williams, John C.; Weijland, Albert; Gonfloni, Stefania; Thompson, Andy; Courtneidge, Sara A.; Superti-Furga, Giulio; Wierenga, Rik K.
SO J. Mol. Biol. (1997), 274(5), 757-775
CODEN: JMOBAK; ISSN: 0022-2836

L11 ANSWER 20 OF 34 CA COPYRIGHT 2001 ACS
PY 1997
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SO EMBO J. (1997), 16(24), 7261-7271
CODEN: EMJODG; ISSN: 0261-4189

L11 ANSWER 21 OF 34 CA COPYRIGHT 2001 ACS
PY 1997
AU Sicheri, Frank; Kuriyan, John
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CODEN: COSBEF; ISSN: 0959-440X

L11 ANSWER 22 OF 34 CA COPYRIGHT 2001 ACS
PY 1997
AU Najmudin, Shabir; Bax, Ben; Guillory, Richard J.
SO J. Biochem., Mol. Biol. Biophys. (1997), 1(2), 73-88
CODEN: JBMBF6; ISSN: 1025-8140

L11 ANSWER 23 OF 34 CA COPYRIGHT 2001 ACS
PY 1997
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SO J. Biol. Chem. (1997), 272(29), 17899-17902
CODEN: JBCHA3; ISSN: 0021-9258

L11 ANSWER 24 OF 34 CA COPYRIGHT 2001 ACS
PY 1997
AU Burton, Elizabeth A.; Hunter, Seija; Wu, Steven C.; Anderson, Steven M.
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CODEN: JBCHA3; ISSN: 0021-9258

L11 ANSWER 25 OF 34 CA COPYRIGHT 2001 ACS
PY 1997
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CODEN: PRCIEI; ISSN: 0961-8368

L11 ANSWER 26 OF 34 CA COPYRIGHT 2001 ACS

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CODEN: NATUAS; ISSN: 0028-0836

L11 ANSWER 27 OF 34 CA COPYRIGHT 2001 ACS
PY 1997
AU Moarefi, Ismail; LeFevre-Bernt, Michelle; Sicheri, Frank; Huse, Morgan; Lee, Chi-Hon; Kuriyan, John; Miller, W. Todd
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CODEN: NATUAS; ISSN: 0028-0836

L11 ANSWER 28 OF 34 CA COPYRIGHT 2001 ACS
PY 1997
AU Anderson, Steven M.; Burton, Elizabeth A.; Koch, Becky L.
SO J. Biol. Chem. (1997), 272(2), 739-745
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L11 ANSWER 29 OF 34 CA COPYRIGHT 2001 ACS
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CODEN: BICHAW; ISSN: 0006-2960

L11 ANSWER 30 OF 34 CA COPYRIGHT 2001 ACS
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T.
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L11 ANSWER 31 OF 34 CA COPYRIGHT 2001 ACS
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L11 ANSWER 32 OF 34 CA COPYRIGHT 2001 ACS
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IN Cambier, John C.; Pleiman, Christopher M.; Clark, Marcus R.
SO PCT Int. Appl., 129 pp.
CODEN: PIXXD2

L11 ANSWER 33 OF 34 CA COPYRIGHT 2001 ACS
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AU Kefalas, P.; Brown, T. R. P.; Brickell, P. M.
SO Int. J. Biochem. Cell Biol. (1995), 27(6), 551-63
CODEN: IJBBFU; ISSN: 1357-2725

L11 ANSWER 34 OF 34 CA COPYRIGHT 2001 ACS
PY 1995
AU Briggs, Scott D.; Bryant, Sophia S.; Jove, Richard; Sanderson, Sam D.; Smithgall, Thomas E.
SO J. Biol. Chem. (1995), 270(24), 14718-24